Åbo Akademi University

Extraction of polymeric galactoglucomannans from spruce wood by pressurised hot water

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Turku/Åbo 2013

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Academic Dissertation

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志不坚者智不达, 言不信者行不果
《墨子•修身》

His wisdom will not be far-reaching whose purpose is not firm.
His action will not be effective whose promises are not kept.
«Micius•Self-cultivation»

To Tingting, Erin and my families

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PREFACE

The work presented in the thesis was carried out in the Laboratory of Wood and Paper Chemistry at Åbo Akademi University under supervision of Docent Andrey Pranovich and Professor (emeritus) Bjarne Holmbom. The work was part of the activities of the Process Chemistry Centre within the Finnish Centre of Excellence Programme (2006 – 2011) by the Academy of Finland, part of the activities within the EPNOE network (European Polysaccharide Network of Excellence), and part of the activities within the project Future Biorefinery of the Metsäklusteri Oy. The work was financed by EPNOE, Process Chemistry Centre and Metsäklusteri Oy, which are acknowledged gratefully.

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LIST OF PUBLICATIONS

This thesis is a summary based on the following publications, which are referred to in the text by their Roman numerals:

I. Tao Song, Andrey Pranovich, Ivan Sumerskiy and Bjarne Holmbom
Extraction of galactoglucomannan from spruce wood with pressurised hot water
Holzforschung 2008, 62 (6), 659 – 666.

II. Tao Song, Andrey Pranovich and Bjarne Holmbom
Characterisation of Norway spruce hemicelluloses extracted by pressurised hot-water extraction (ASE) in the presence of sodium bicarbonate
Holzforschung 2011, 65 (1), 35 – 42.

III. Tao Song, Andrey Pranovich and Bjarne Holmbom
Effects of pH control with phthalate buffers on hot-water extraction of hemicelluloses from spruce wood
Biores. Technol. 2011, 102 (22), 10518 – 10523.

IV. Tao Song, Andrey Pranovich and Bjarne Holmbom
Hot-water extraction of ground spruce wood of different particle size
BioResources 2012, 7 (3), 4214 – 4225.

V. Tao Song, Andrey Pranovich and Bjarne Holmbom
Separation of polymeric galactoglucomannans from hot-water extract of spruce wood
Biores. Technol. 2013, 130, 198 – 203.

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Model for degradation of galactoglucomannan in hot water extraction conditions
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CONTRIBUTIONS OF THE AUTHOR

Paper I: The author prepared all the samples, did all the experimental work, interpreted and finalised the manuscript together with the co-authors.

Papers II, III, IV and V: The author planned the experimental work together with the co-authors, prepared all the samples, did all the experimental work, interpreted the results, wrote the first draft of the manuscripts and finalised them with the co-authors.

Paper VI: The author planned the experimental work together with the co-authors, prepared all samples, and did the experimental work.

OTHER PUBLICATIONS RELATED TO THE TOPIC

1. Tao Song, Andrey Pranovich, Ivan Sumerskiy and Bjarne Holmbom
Extraction of galactoglucomannan from spruce wood with pressurised hot water
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2. Tao Song, Andrey Pranovich and Bjarne Holmbom
Pressurised water extraction of galactoglucomannan from spruce wood with addition of Sodium Bicarbonate
In: *Proceedings of the 15th International Symposium on Wood, Fibre and Pulping Chemistry*, June 2009, PTF, Oslo, Norway, poster presentation.

3. Tao Song, Andrey Pranovich and Bjarne Holmbom
Pressurised water extraction of galactoglucomannan from spruce wood with addition of sodium bicarbonate
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4. Tao Song, Andrey Pranovich and Bjarne Holmbom
Pressurised hot water extraction of galactoglucomannans from spruce wood with addition of phthalate buffers
In: *Proceedings of the 11th European Workshop on Lignocellulosics and Pulp*, August 2010, vTI, Hamburg, Germany, oral presentation, pp. 133 – 136.

5. Tao Song, Andrey Pranovich and Bjarne Holmbom
Effects of pH control with phthalate buffers on hot-water extraction of hemicelluloses from spruce wood
In: *Proceedings of the 16th International Symposium on Wood, Fibre and*

Pulping Chemistry, June 2011, CTAPI, Tianjin, China, oral presentation, pp. 408 – 412.

ABBREVIATIONS

Ara	Arabinose
ASE	Accelerated solvent extractor/extraction
Gal	Galactose
GalA	Galacturonic acid
GC	Gas chromatography
GGM	Galactoglucomannan
Glc	Glucose
GlcA	Glucuronic acid
HPLC	High-performance liquid chromatography
HPSEC-MALLS	High-performance size-exclusion chromatography multi-angle laser light scattering detector
LLRS	Lignin and lignin-related substances
Man	Mannose
4-*O*-MeGlcA	4-*O*-methylglucuronic acid
MTBE	Methyl *tertiary*-butyl ether
Mw	Average weight molar mass
NMR	Nuclear magnetic resonance spectroscopy
Rha	Rhamnose
TDS	Total dissolved solids
TMP	Thermo-mechanical pulp
Xyl	Xylose

ABSTRACT

The major type of non-cellulosic polysaccharides (hemicelluloses) in softwoods, the partly acetylated galactoglucomannans (GGMs), which comprise about 15% of spruce wood, have attracted growing interest because of their potential to become high-value products with applications in many areas. The main objective of this work was to explore the possibilities to extract galactoglucomannans in native, polymeric form in high yield from spruce wood with pressurised hot-water, and to obtain a deeper understanding of the process chemistry involved. Spruce (*Picea abies*) chips and ground wood particles were extracted using an accelerated solvent extractor (ASE) in the temperature range 160 – 180°C. Detailed chemical analyses were done on both the water extracts and the wood residues.

As much as 80 – 90% of the GGMs in spruce wood, i.e. about 13% based on the original wood, could be extracted from ground spruce wood with pure water at 170 – 180°C with an extraction time of 60 min. GGMs comprised about 75% of the extracted carbohydrates and about 60% of the total dissolved solids. Other substances in the water extracts were xylans, arabinogalactans, pectins, lignin and acetic acid. The yields from chips were only about 60% of that from ground wood. Both the GGMs and other non-cellulosic polysaccharides were extensively hydrolysed at severe extraction conditions when pH dropped to the level of 3.5.

Addition of sodium bicarbonate increased the yields of polymeric GGMs at low additions, 2.5 – 5 mM, where the end pH remained around 3.9. However, at higher addition levels the yields decreased, mainly because the acetyl groups in GGMs were split off, leading to a low solubility of GGMs. Extraction with buffered water in the pH range 3.8 – 4.4 gave similar yields as with plain water, but gave a higher yield of polymeric GGMs. Moreover, at these pH levels the hydrolysis of acetyl groups in GGMs was significantly inhibited. It was concluded that hot-water extraction of polymeric GGMs in good yields (up to 8% of wood) demands appropriate control of pH, in a narrow range about 4. These results were supported by a study of hydrolysis of GGM at constant pH in the range of 3.8 – 4.2 where a kinetic model for degradation of GGM was developed.

The influence of wood particle size on hot-water extraction was studied with particles in the range of 0.1 – 2 mm. The smallest particles (< 0.1 mm) gave 20 – 40% higher total yield than the coarsest particles (1.25 – 2 mm). The difference was greatest at short extraction times. The results indicated that extraction of GGMs and other polysaccharides is limited mainly by the mass transfer in the fibre wall, and for coarse wood particles also in the wood matrix. Spruce sapwood, heartwood and thermomechnical pulp were also compared, but only small differences in yields and composition of extracts were found.

Two methods for isolation and purification of polymeric GGMs, i.e. membrane filtration and precipitation in ethanol-water, were compared. Filtration through a series of membranes with different pore sizes separated GGMs of different molar masses, from polymers to oligomers. Polysaccharides with molar mass higher than 4 kDa were precipitated in ethanol-water. GGMs comprised about 80% of the precipitated polysaccharides. Other polysaccharides were mainly arabinoglucuronoxylans and pectins. The ethanol-precipitated GGMs were by ^{13}C NMR spectroscopy verified to be very similar to GGMs extracted from spruce wood in low yield at a much lower temperature, 90°C.

The obtained large body of experimental data could be utilised for further kinetic and economic calculations to optimise technical hot-water extraction of softwoods.

REFERAT

Ved innehåller förutom cellulosa också andra polysackarider, så kallade hemicellulosor. Omkring 15 % av granveden består av partiellt acetylerade galaktoglukomannaner (GGM), vilka har rönt ett stigande intresse p.g.a. deras stora potential att bli högvärdiga produkter med användning inom många områden. Huvudsyftet med detta arbete var att utforska möjligheterna att extrahera GGM i nativ, högmolekylär form med ett högt utbyte ur granved med hett vatten under tryck samt att erhålla en djupare förståelse av den processkemi som här förekommer. Flis och malda vedpartiklar av gran (*Picea abies*) extraherades med en ASE-apparat (Accelerated Solvent Extractor) i temperaturområdet 160-180 °C. Ingående kemiska analyser utfördes både på vattenextrakt och på extraherad ved.

Så mycket som 80-90 % av granvedens GGM, d.v.s. omkring 13 % av veden, kunde extraheras med rent vatten vid 170-180 °C vid en extraktionstid på 60 min. GGM utgjorde omkring 75 % av de extraherade kolhydraterna och omkring 60 % av hela mängden utlöst material. Andra substanser i vattenextrakten utgjordes av xylaner, arabinogalaktaner, pektiner, lignin och ättikssyra. Både GGM och andra polysackarider hade hydrolyserats i hög grad under stränga extraktionsförhållanden vilket ledde till att pH sjönk till en nivå av 3,5. Utbytet ur flis var bara omkring 60 % av utbytet ur mald ved.

Tillsats av 2,5-5 mM natriumbikarbonat ökade utbytet av polymert GGM, då slut-pH stannade omkring 3,9. Vid högre tillsatsnivåer minskade emellertid utbytet, huvudsakligen p.g.a. att acetylgrupperna i GGM spjälkades bort, vilket ledde till en låg löslighet för GGM. Extraktion med buffrat vatten i pH-området 3,8-4,4 gav liknande utbyten som rent (icke-buffrat) vatten men gav ett högre utbyte av polymert GGM. Dessutom inhiberades hydrolysen av acetylgrupperna betydligt vid dessa pH-nivåer. Slutsatsen som drogs var att extraktion av polymert GGM med hett vatten i höga utbyten (upp till 8 % av veden) kräver en god kontroll av pH eftersom extraktionen är optimal endast i ett smalt område kring 4. Dessa resultat stöddes av en studie av hydrolys av GGM vid konstant pH i området 3,8-4,2, där en kinetisk modell för nedbrytningen av GGM utvecklades.

Inverkan av storleken hos vedpartiklarna på hetvattenextraktionen undersöktes i området 0,1-2 mm. De minsta partiklarna (>0.1 mm) gav ett 20-40 % högre totalutbyte än de grövsta partiklarna. Skillnaden var störst vid korta extraktionstider. Resultaten tyder på att extraktion av GGM och andra polysackarider främst begränsas av massöverföringen i fiberväggen och för grova partiklar därtill också av vedmatrisen. Splintved, kärnved och termomekanisk massa jämfördes också, men endast små skillnader i utbyten och extraktens sammansättning erhölls.

Två metoder för isolering och rening av GGM, d.v.s. membranfiltrering och utfällning i etanol-vatten, jämfördes. Filtrering med en serie membraner av olika porstorlek separerade GGM med olika molmassor, från polymerer till monomerer. Polysackarider med molmassor högre än 4 kDa utfälldes i etanol-vatten. GGM utgjorde omkring 80 % av de utfällda polysackariderna. De andra polysackariderna var arabinoglukuronoxylaner och pektiner. Analyser med ^{13}C NMR spektroskopi bekräftade att GGM utfällda i etanol var mycket lika det GGM som hade extraherats ur granved vid en mycket lägre temperatur, 90 °C.

Den stora mängden experimentella data som erhölls i arbetet kunde utnyttjas för vidare kinetiska och ekonomiska beräkningar för att optimera hetvattenextraktionen av GGM ur barrved.

KEYWORDS

acetyl groups; ASE; biomass; buffer; galactoglucomannan; hemicelluloses; hot-water extraction; lignin; membrane filtration; molar mass; non-cellulosic carbohydrates; pressurised extraction; *Picea abies*; solvent precipitation; spruce wood; wood biorefinering; xylan.

1. INTRODUCTION

1.1 Biomass and biorefinering

Biomass is a large renewable resource which plays a growing global role because of its sustainable character. Biomass can be utilised for production of energy, fuels, materials and chemicals, commonly called bioenergy, biofuels, biomaterials and biochemicals.

Biomass is one of humanity's earliest sources of energy, mainly used in the form of wood for heating and cooking. Nowadays, biomass energy is wanted in the form of biofuels, such as bioethanol and biodiesel. The biofuels are preferred over conventional fuels, i.e. coal, oil and petroleum, because biomass is a renewable resource and produces fewer CO_2 emissions. Biomaterials and biochemicals, also called green materials and chemicals have not only become more and more important in scientific research, but have also found special applications.

Currently, the main sources of biofuels and biochemicals are agricultural crops. For example, bioethanol is produced mainly from corns, sugarcanes and beans. Carbohydrates, such as starch, fructose and sucrose, are produced from potatoes, sugarcanes and beets. These crops are also used as food in people's daily lives, and their use for biofuel and biochemical production can lead to increase the price of crops-derived food, and even cause a food crisis in the world. Therefore, wood and other biomass that are not used for food production have received increasing interest as raw materials for so-called biorefineries.

Biorefinery is an overall concept of integrated and diversified processing plants where biomass feedstocks are fractionated and converted into a wide range of valuable products, much like in petroleum refineries. Integrated biorefineries are processing facilities that extract carbohydrates, oils, lignin, and other materials from biomass, and convert them into products such as fuels, high-value chemicals and other materials, with a zero waste approach (Ohara 2003; Kamm, B. and M. 2004; Werpy et al., 2004; Kamm et al., 2006;

Wright et al., 2006). Extraction of the plant raw materials is usually the most crucial step in the production of novel products from biomass. A fully integrated extraction system can transform all the raw materials into useful, high-value products.

1.2 Wood biorefinering

Woody biomass is the most abundant renewable material, accounting for about 50% of all the biomass in the world (Claassen et al., 1999; Wingren et al., 2003). Woody biomass is also the most abundant source of carbohydrates worldwide. Wood contains polysaccharides, consisting primarily of cellulose and non-cellulosic polysaccharides, i.e. hemicelluloses and pectins, with varying proportions in different wood species, plus lignin and smaller amounts of other materials (Sjöström, 1993). Today, wood is mainly used in pulping and papermaking, energy generation by combustion, and as building material. In recent years, renewable wood-derived polysaccharides have attracted growing interest because of their great potential as new raw materials for the production of specialty materials and chemicals, other than pulp and paper (Ragauskas et al., 2006; Willför et al., 2008). Non-cellulosic polysaccharides are possible precursors to different high-value chemicals and materials. Non-cellulosic polysaccharides consist of many different types of heteropolysaccharides, mainly xylans in hardwood such as birch, and galactoglucomannans (GGMs) in softwood such as spruce.

Many techniques have been studied for extraction of non-cellulosic carbohydrates from wood, such as steam explosion (Palm and Zacchi 2004; Li et al., 2005), treatment with alkali (Al-dajani and Tschirner 2008, 2010) or diluted acid (Söderström et al., 2003; Vanessa et al., 2008) and hot-water extraction, also named autohydrolysis or hydrothermolysis (Lai 2001; Bonn et al., 1983). In these studies, mostly hardwoods have been investigated because they are under-utilised in many countries (Karlsson et al., 2006; van Heiningen 2006; Leschinsky et al., 2009; Liu and Amidon 2007; Al-dajani and Tschirner 2008; Chirat et al., 2012). However, softwoods, such as pine wood (Casebier et al., 1969; Yoon and van Heiningen 2008; Yoon et al., 2008) and spruce wood (Lundqvist et al., 2002 and 2003), have also been investigated. Non-cellulosic carbohydrates have been extracted from spruce

wood with water at temperatures below 100°C (Örså et al., 1997), by microwave heat-fractionation (Lundqvist et al., 2002 and 2003) and alkaline extraction (Capek et al., 2000). Some part of non-cellulosic carbohydrates of spruce can be isolated as lignin-carbohydrate-complexes (LCCs) (Lawoko et al., 2006).

Many factors have been studied to develop the extraction of non-cellulosic carbohydrates from wood. Lundqvist et al., (2002) compared spruce non-cellulosic carbohydrate extraction by impregnating chips with different solutions. Influence of impregnation time and chip size has also been studied (Ballesteros et al., 2000; Monavari et al., 2009). In most studies on hot-water extraction of wood, the ultimate goal has been to produce sugars and further biofuels, such as bioethanol (Ragauskas et al., 2006 and 2007; Liu and Amidon 2007).

Only more recently, the concept of structure-preserving extraction of wood, has emerged. The concept aims at extraction of the non-cellulosic polysaccharides in native, undegraded form, avoiding hydrolysis as much as possible. The extracted non-cellulosic polysaccharides could find uses in a variety of applications such as in food, health, papermaking, textile and cosmetic industries (Ebringerová et al., 2005; Kollárová et al., 2006; Mikkonen et al., 2008; Willför et al., 2008; Xu et al., 2009a). Non-cellulosic polysaccharides can be used directly for novel industrial applications as biopolymers and hydrogels (Ebringerová et al., 1994; Gabrielii et al., 2000; Hartman et al., 2006; Mikkonen et al., 2008), or after hydrolysis serve as a source of sugars for fermentation to fuel, such as bioethanol, or chemicals. Sugars derived from non-cellulosic polysaccharides have also a large potential for the production of various sugar-based chemicals, including so-called bioplastics (Werpy et al., 2004).

1.3 Wood structure and chemistry

Wood is a very heterogeneous natural material, where the structural components, i.e. cellulose, non-cellulosic polysaccharides (hemicelluloses and pectins) and lignin, are dominating. The structural components typically comprise 97 – 99% of the wood material. The polysaccharides range in

structure from linear to highly branched. Structural polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even soluble in water (Varki et al., 2008).

There are also small amounts of non-structural compounds, commonly collectively called extractives in wood. They consist of a wide range of low-molecular-mass substances (Fengel and Wegener 1984) such resin, fats, phenols and carbohydrates. Some extractives are energy sources for the wood cells and take part in the catalysis of biosynthetic processes. Extractives can also protect the wood against microbiological damage or attack by herbivores attacks. The content of extractives in wood is typically 3 – 5%, but the content can be different in different wood species, different parts of the wood, and even at different seasons.

There are about 100,000 wood species, and the majority of wood species grow in tropical regions of the world. In Finland, about 70% of the land area is covered with forest. The wood species that dominate in Finland are pine (*Pinus sylvestris*, 50%), spruce (*Picea abies*, 31%), and birch (*Betula pendula*, 16%). All wood species can be classified either as hardwoods (angiosperms) or as softwoods (gymnosperms).

1.3.1 Hardwoods and softwoods

Hardwoods are trees of the deciduous class, usually with broad leaves, while the trees classified as softwoods (coniferous) have needle-like or scale-like leaves.

The chemical compositions of softwoods and hardwoods are different (Table 1). Softwoods contain more lignin but less cellulose than hardwoods. The non-cellulosic polysaccharides are also different. Softwoods contain predominantly (galacto)glucomannans, while hardwoods contain mainly xylans.

Table 1. Typical compositions of hardwood and softwood (Sjöström 1993).

	Hardwood (% of wood)	Softwood (% of wood)
Cellulose	44±6	38±5
Hemicelluloses	24±6	25±4
Glucomannans	2.5±1	17±3
Xylans	23±7	8±3
Other polysaccharides	3±1	6±3
Lignin	24±3	34±7
Extractives	3±2	5±3

1.3.2 Sapwood and heartwood

In the cross section of the stem, heartwood and sapwood is usually clearly distinguished. Sapwood is formed by a thin layer of living cells known as the cambium, which produces bark cells to the outside and wood cells to the inside. Sapwood contains a variety of cell types, most of which are dead tracheids and some of which are living parenchyma cells. As the sapwood parenchyma cells age and die, heartwood is formed. In softwood, the pits will be closed, or aspirated, when heartwood is formed. The relative amounts of sapwood and heartwood can vary greatly among trees, depending on age, species, and growing conditions. There are also some chemical and physical differences between sapwood and heartwood. Generally, heartwood contains less cellulose and non-cellulosic polysaccharides (Timell 1986; Fengel and Wegener 1984; Chen 1991; Shupe et al., 1997). Concerning non-cellulosic polysaccharides, only small differences have been observed between heartwood and sapwood (Rogers and Perkins 1968; Holmbom et al., 2000; Willför et al., 2005a and 2005b). However, in larch species heartwood contains much more arabinogalactans than sapwood. There is no starch in heartwood.

1.3.3 Cell wall layers

Wood cell walls consist mainly of cellulose, non-cellulosic carbohydrates and lignin. The wood cells have a thick fibre wall built up of different layers (Figure 1). From the outside to the inside the layers are middle lamella (M), primary wall (P) and a multi-layered secondary wall. The chemical

composition is different in different layers (Sjöström 1993).

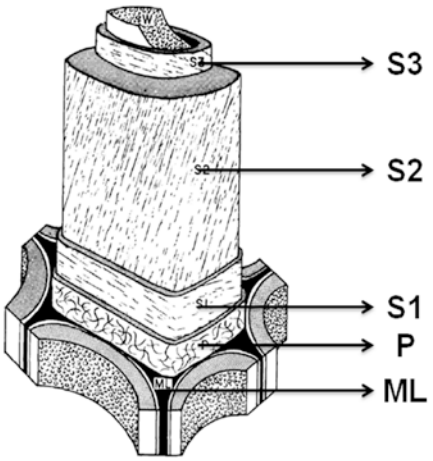


Figure 1. Schematic presentation of the cell wall layers. ML, middle lamella; P, primary cell wall; S1, S2 and S3, layers of secondary cell wall. Picture redrawn from Côté (1967).

The middle lamella is the outermost cell wall layer providing the adhesion between fibres. The middle lamella together with the primary cell wall are rich in pectins and lignin (Timell 1967). In the middle lamella, lignin has the highest concentration and cements the fibres together. The remaining part of lignin occurs throughout the secondary wall.

The primary cell wall is thin (0.1 – 0.2 µm) and contains a randomly and loosely organized network of cellulose microfibrils. Non-cellulosic carbohydrates and lignin also occur in the primary cell wall (Meier 1962).

The secondary wall is formed when the cell wall is thickening. It is composed of three layers: the thin S1 (0.1 – 0.3 µm), the thick S2 (1 – 5 µm) and the thin S3 (ca. 0.1 µm). In the S2-1ayer, the main portion of the secondary wall, the microfibrils are oriented almost parallel to the fibre axis. Most of the hemicelluloses are located in the secondary cell wall. Galactoglucomannans (GGMs) and arabinoglucuronoxylans (AGXs), the major non-cellulosic polysaccharides in the secondary cell wall of softwoods, also occur as minor components in the primary cell wall (Meier 1962). The amounts of xylans have been found to be the lowest in the middle layer of the secondary cell

wall (S2) and considerably higher in the S1 and S3 layers. The distribution of GGMs is opposite to that of xylans, with most of GGMs located in the S2 layer and less in the S1 and S3.

1.3.4 Cellulose

Cellulose is the main constituent of wood and is located predominantly in the secondary cell wall. In most wood species, 40 – 45% of the dry substance is cellulose. Cellulose is a homopolysaccharide composed of β-D-glucopyranose units linked together by (1→4)-glycosidic bonds which are more stable than hemicellulose glycosidic bonds. Native cellulose molecules are completely linear and have a strong tendency to form intra- and intermolecular hydrogen bonds (Sjöström 1993). Bundles of cellulose molecules are aggregated together in the form of microfibrils, in which highly ordered (crystalline) regions alternate with less ordered (amorphous) regions. The crystalline regions and the strong hydrogen bonds make the cellulose resistant towards dissolution and reactions. It is believed that native wood cellulose molecules in the secondary wall consist of about 10,000 glucose units, and are monodisperse. On the other hand, the native cellulose molecules in the primary cell wall are polydisperse and have a lower average molar mass.

1.3.5 Non-cellulosic polysaccharides in spruce wood

Galactoglucomannans (GGMs)

Galactoglucomannans, also called glucomannans (GMs) for short, are built up of anhydro-mannose, -glucose and -galactose units. GGMs are the principal non-cellulosic polysaccharides in softwoods, amounting to 14 – 20% of wood (Willför et al., 2005a; Xu et al., 2009b). The amount of GGMs differ between wood species and also between earlywood and latewood as well as between normal and compression wood (Timell 1967 and 1986; Fengel and Wegener 1984; Bertaud and Holmbom 2004; Willför et al., 2005a). GGMs in wood have been reported to have an approximate degree of polymerisation of 100 – 150, corresponding to a molar mass of 16 – 24 kDa (Timell 1967). The water-soluble acetylated GGMs dissolved in thermomechanical pulping (TMP) consist of a backbone of β-(1→4)-D-Manρ and β-(1→4)-D-Glcρ units at a ratio of 10:1.9 – 2.6 with side units of α-(1→

6)-D-Gal*p* (Figure 2) (Willför et al., 2003; Hannuksela and Hervé du Penhoat 2004). The mannopyranosyl units are acetylated at C-2 and C-3 with a degree of acetylation of 0.28 – 0.37. Their molar mass for water-soluble spruce GGMs from TMP has been reported to be 20 – 60 kDa. The degree of acetylation and the number of galactose units as side groups play a key role for the water solubility of GGMs. The removal of these groups decreases the GGMs solubility considerably (Timell 1965; Hannuksela et al., 2003). At alkaline conditions, both acetyl and galactose side groups are easily cleaved (Sjöström 1993; Capek et al., 2000). However, at room temperature and pH about 2, the acetyl-GGMs of spruce are stable for a long time (Xu et al., 2009b).

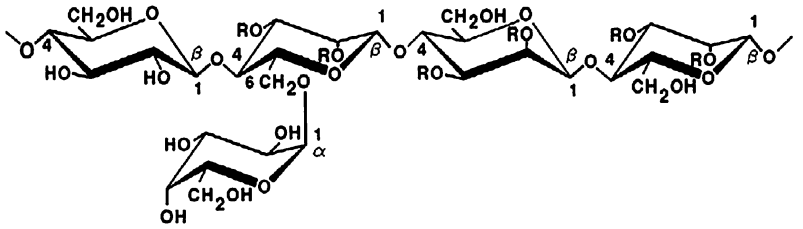


Figure 2. Principal structure of galactoglucomannan, R=CH_3CO or H (Sjöström 1993).

GGMs in wood and pulps have been of interest primarily because of their importance in pulping and papermaking. Only recently, research has been focused on the extraction of GGMs from wood as part of biorefinery concepts.

Arabinoglucuronoxylans (xylans)

Softwoods contain 5 – 10% arabino-4-*O*-methylglucuronoxylans (xylans) with a backbone of (1→4)-linked *β*-D-xylopyranose units and side groups at C-2 of 4-*O*-methyl-*α*-D-glucuronic acid groups on the average of two residues per ten xylose units (Figure 3) (Fengel and Wegener 1984, Sjöström 1993, Shimizu 2001).

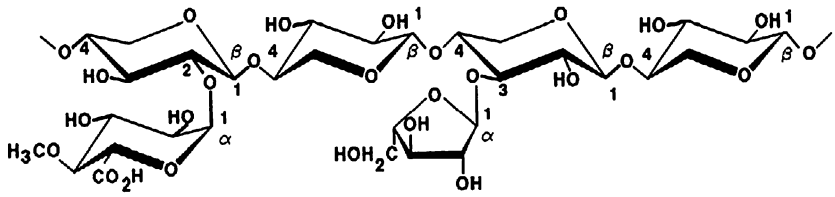


Figure 3. Principal structure of arabinoglucuronoxylans (Sjöström 1993).

In addition, side groups of α-L-arabinofuranose units, on the average 1.3 residues per ten xylose units, also occur. The arabinose units are easily split off at acidic conditions.

Pectins and other minor polysaccharides

Pectins, also called polygalacturonans, are anionic polysaccharides which primarily consist of galacturonic acid and rhamnose units in a molar ratio of GalA:Rha = 6:1. These polysaccharides give an anionic charge to the fibre. Pectins are partly dissolved in circulation waters of mechanical pulping plants. Especially at alkaline conditions, the dissolved pectins can, due to their anionic character, interact with and consume added process chemicals. In the heartwood of larch, quite large amounts of arabinogalactans are found. Smaller amounts are found in compression wood and heartwood of other softwood species, including spruce and pine (Willför et al., 2002). About 90% of the arabinogalactans in larch are located outside the cell wall (Côté et al., 1966), primarily filling the lumen of tracheids that are close to ray cells. Arabinogalactans are water-soluble due to their highly branched structure, consisting of a backbone of β-(1→3) linked galactose and side chains of β-(1→6) linked galactose, arabinose and glucuronic acid (Fengel and Wegener 1984; Willför et al., 2002).

1.3.6 Lignin

Lignin is the three-dimensional, amorphous polymer that glues the fibres together in the middle lamella and the fibrils together in the secondary cell wall (Sjöström 1993). Lignin gives the tree and the wood its unique mechanical strength. The building blocks of lignin, the phenylpropane units, are randomly linked to each other and by ether and carbon-to-carbon bonds (Figure 4).

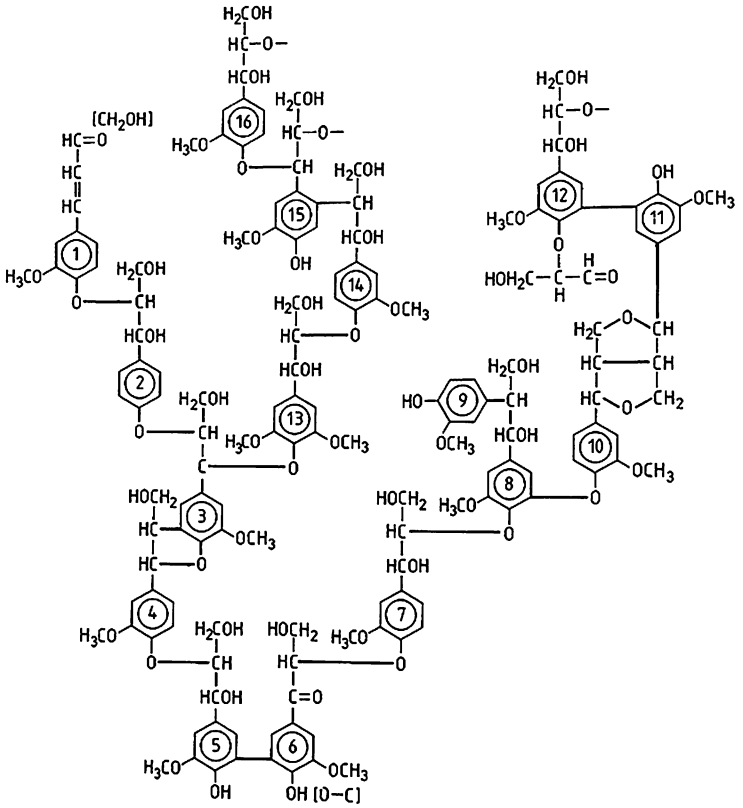


Figure 4. A structural segment of softwood lignin (Adler 1977).

The phenylpropane units are of *p*-coumaryl, coniferyl and sinapyl alcohol. Different plant types produce these phenylpropane units in different ratios. Hardwood contains more sinapyl alcohol, grasses more *p*-coumaryl alcohol, while coniferyl alcohol dominates in softwoods.

1.3.7 Distribution of cellulose, hemicelluloses and lignin in the cell wall

A distribution model of cellulose, hemicellulose and lignin in the cell wall layers is shown in Figure 5. Both hemicelluloses (polyoses) and less ordered cellulose molecules enclose the highly ordered cellulose domains. Some hemicellulose molecules are additionally deposited within the lignin layer.

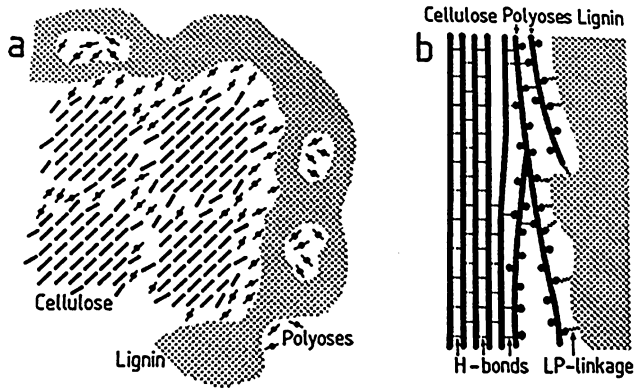


Figure 5. Model of cellulose, hemicelluloses (polyoses) and lignin distribution in the S2 cell wall, in transverse (a) and longitudinal view (b) (Fengel and Wegener 1984).

It is believed that the orientation of the hemicelluloses molecules is in parallel to the cellulose microfibrils (Page 1976; Åkerholm and Salmén 2001; Stevanic and Salmén 2009). The hemicelluloses in the cell wall are linked to cellulose fibrils by hydrogen bonds, while lignin in the cell wall is linked with hemicelluloses also by some covalent bonds (Fengel and Wegener 1984).

Wood is a porous material with small pores in the cell walls (Berthold and Salmén 1997; Kojiro et al., 2010). Untreated wood contains mainly pores smaller than 2.0 nm, named micropores, and pores ranging from 2 to 50 nm, named mesopores (Kojiro et al., 2010). Untreated thermomechanical pulp (TMP) fibres, which in structure are similar to native wood fibres, contain mainly micropores (Figure 6).

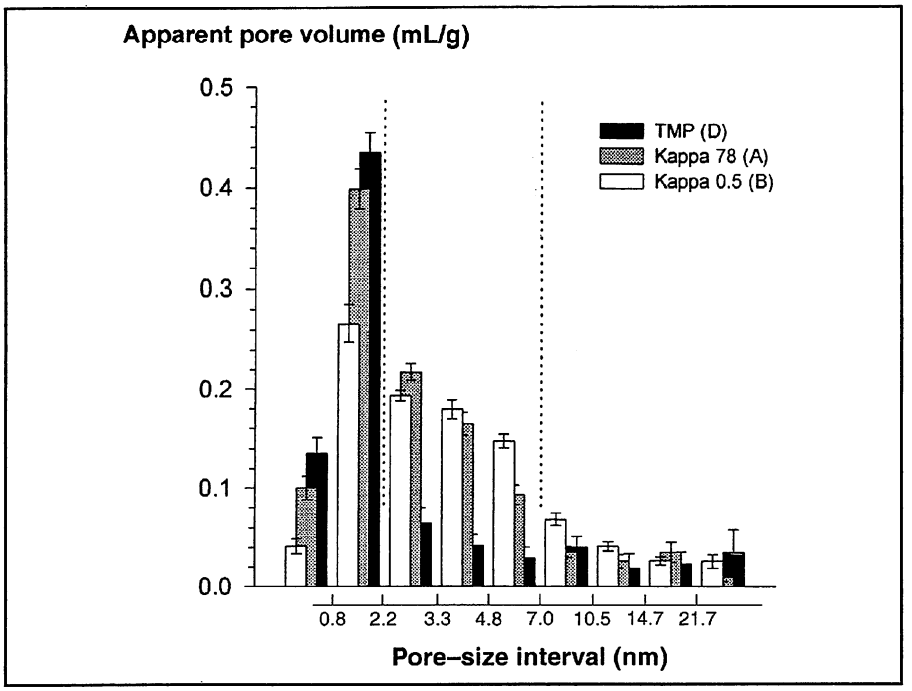


Figure 6. The apparent pore volume in different pore-size intervals for three pulps: an unbleached kraft pulp (pulp A), a bleached kraft pulp (pulp B) and a TMP (pulp D). The apparent pore-size distribution changes as lignin is removed gradually from the pulp (Berthold and Salmén 1997).

Micropores smaller than 2.2 nm were found to contribute with about 70% of the total pore volume. The apparent pore size was determined by inverse size-exclusion chromatography with pullulans (glucans with α-1, 4 and α-1, 6 bonds) of different molar masses. It was found that micropores (<2.2 nm) allow penetration of pullulans with a molar masses up to 5.4 kDa, and pores of 10.5 nm even up to 100 kDa molecules.

During pulping when lignin and hemicelluloses are removed the pore structure will change dramatically (Treimanis 1996). Unbleached kraft pulp was found to contain much more pores in the 2 – 7 nm range than TMP (Berthold and Salmén 1997). Bleached pulp had only a small volume in micropores, and a much larger volume in the 4.8 – 10.5 nm interval. However, little is known about the change in pore structure and size distribution during hot-water extraction, where mainly hemicelluloses and only little lignin are

removed.

1.4 Thermomechanical pulp (TMP)

Thermomechanical pulp (TMP) is still today an important pulp type, especially in the Nordic countries and Canada. In the TMP process, wood logs are first stripped of their bark and then cut into small chips. The pulp is made by heating the chips with pressurised steam and separating the fibres in a pressurised refiner (Fengel and Wegener 1984). The optimum refining temperature in the production of TMP for paper furnish is 115 – 130°C. At this temperature, the heat and moisture soften the wood lignin, which results in efficient separation of undamaged fibres. The separation of the fibres takes place mainly in the outer secondary wall (S1) and the primary wall of the fibres, while the middle lamella remains relatively unchanged.

During TMP refining, about 5 – 10% of GGMs in the wood are dissolved in the process water (Thornton 1993). In TMP processing, different parts of the fibre layers (P and S1) are peeled off and expose the secondary cell wall on the surface. This enables polysaccharides to be released to process waters. Cracks are also formed in the fibres.

1.5 Extraction

Various extraction techniques are commonly used prior to instrumental analysis in chemical laboratories. There are two main basic extraction techniques: liquid-liquid and solid-liquid extraction.

1.5.1 Liquid-liquid extraction

Liquid–liquid extraction is also known as solvent extraction. The separation of compounds is based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent.

1.5.2 Solid-liquid extraction

Solid-liquid extraction is dissolution of compounds from a solid material into a liquid phase.

Solid-liquid extraction comprises three phases:

a) Diffusion of the solvent through the pores into the solid material
b) The solvent dissolves the solutes (i.e. transfer the solute to the liquid phase)
c) Transfer of the solutes out from the porous solid material matrix to the main bulk of the solution

Extraction of non-structural components from wood

Lipophilic extractives, e.g. resin, fat and waxes, can be extracted directly by non-polar solvents such as hexane, diethyl ether and MTBE, while hydrophilic extractives, e.g. phenols, sugars and inorganic salts, can be extracted by polar solvents such as acetone, ethanol and water. Arabinogalactans, present especially in the heartwood of larch, are easily dissolved in water, and extracted from the lumen of tracheids that are close to ray cells (Côté et al., 1966).

Extraction of structural components from wood at acidic conditions

The structural components (cellulose, non-cellulosic polysaccharides and lignin) in wood are insoluble in water at mild conditions, except for some branched hemicelluloses, and pectins with low-molar-mass. However, cellulose, hemicelluloses and lignin are all sensitive to thermal and chemical degradation, and hemicelluloses are the most sensitive (Levan et al., 1990; Winandy 1995). During thermal-chemical pretreatment the side groups of hemicellulose are usually cleaved first, followed by cleavage of the hemicellulose backbone (Sweet and Winandy 1999).

Autohydrolysis of the amorphous hemicelluloses is the dominating reaction during pressurised hot-water extraction. Hemicelluloses are the major components in the extracts, appearing mainly in oligo- and mono- form (Garrote et al., 1999; Carvalheiro et al., 2004). Cellulose and lignin are not significantly solubilized.

Autohydrolysis of structural wood compounds during hot-water extraction is a process similar to dilute acid depolymerisation which is catalysed by

hydronium ions (H_3O^+). Water is the only reactant in the initial stage of extraction. In this stage, hydronium ions, the catalysts of hydrolysis, are coming from water auto-ionization at the high temperature. The presence of hydronium ions leads to depolymerisation of non-cellulosic carbohydrates, such as acetyl-GGMs in spruce wood, by hydrolysis of both acetyl groups and glycosidic linkages. Hydrolysis of uronic acids, mainly from pectins, can also occur in this stage, simultaneously with cleavage of acetyl groups (Carrasco et al., 1987). Despite their resistance to hydrolysis, uronic acids may also contribute to formation of hydronium ions (Conner 1984) but their role in hydrolysis is still not completely understood.

Acid hydrolysis of glycosidic bonds in polysaccharides proceeds in three steps (Figure 7).

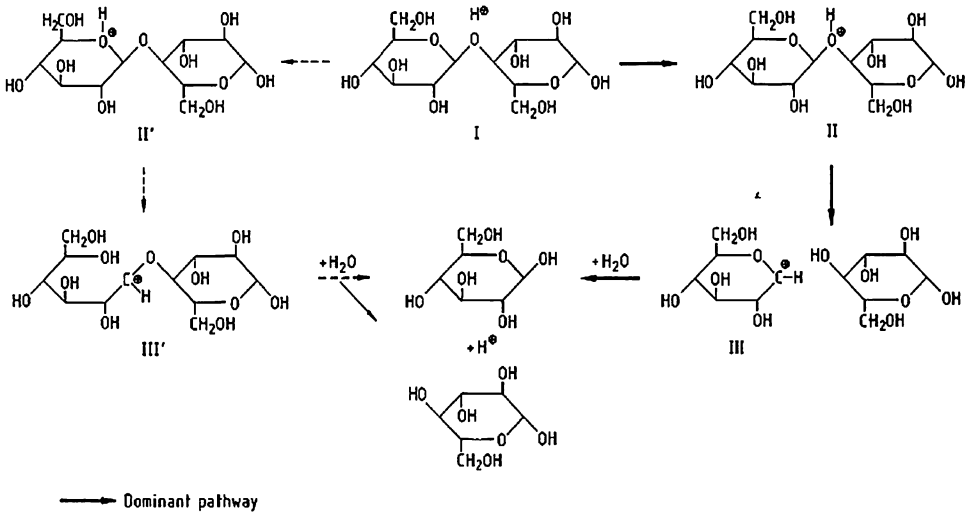


Figure 7. Mechanism of acidic hydrolysis of glycosidic linkages (Fengel and Wegener 1984).

The hydrolysis rate of glycosidic bonds is dependent on the conformation of the sugar units, particularly of the half-chair conformation formed after the protonation at the glycosidic oxygen atom. Generally, the acid hydrolysis rates are faster for α-forms than for β-forms and also faster for pentose units than for hexose units. Accordingly, the hydrolysis rates of the important sugars in wood polysaccharides were found to be similar to *β*-D-glucose:

β-D-mannose: β-D-galactose: β-D-xylose = 1:3:4 – 5:4 – 6 (Fengel and Wegener 1984). Thus, the bonds in cellulose are more stable than bonds in GGMs, which in turn are more stable than bond between xylose units in xylans.

GGMs are hydrolysed even at temperatures of 70 – 90°C when pH is 2 or lower (Xu et al., 2008, Kusema et al., 2013). The hydrolysis is independent on the type of acid. GGM hydrolysis occurs rather randomly. In the beginning, the release of the side unit galactose was slightly faster than those of mannose and glucose constituting the main chain. The activation energy for acid hydrolysis of spruce GGMs was determined to be 150 kJ/mol (Xu et al., 2008).

The acid hydrolysis and deacetylation reactions of Ac-GGMs are outlined in Figure 8.

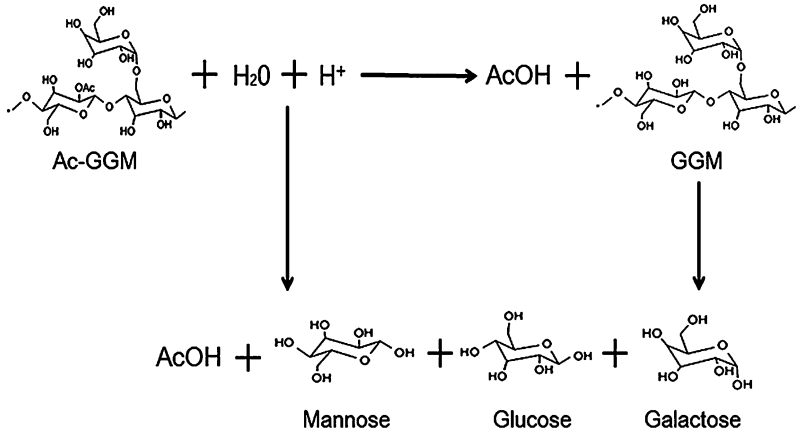


Figure 8. Deacetylation and acid hydrolysis of Ac-GGMs in hot-water extraction.

Xylans are hydrolysed more easily than to GGMs in spruce wood. The acetyl groups are essential for the water solubility of GGMs (Timell 1965) and hydrolysis of acetyl groups dramatically lowers the solubility of GGMs (Hannuksela et al., 2002) in water. Deacetylated GGMs can even be sorbed back to the fibres.

Extended hot-water extraction of wood produces mainly mixtures of

oligosaccharides, monosaccharides and acetic acid. Other extracted substances from structural compounds are lignin fragments and cellulose-derived glucose units. Furan derivatives, e.g. furfural (Dunlop 1948) and hydroxymethylfurfural (Ulbricht et al., 1984) can be formed at severe conditions (Figure 9).

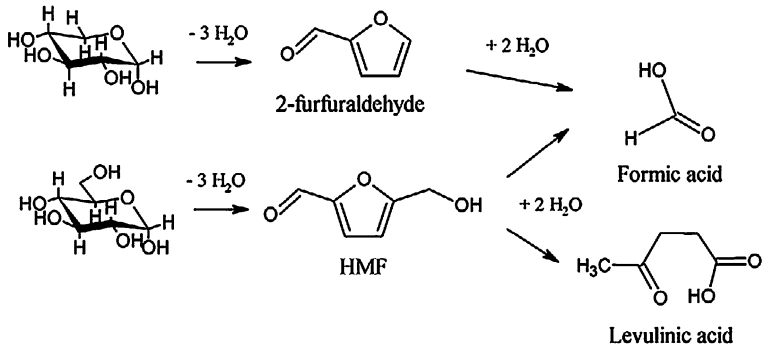


Figure 9. Pathways for formation of acid degradation products from glucose and xylose (Pedersen and Meyer 2010).

The extraction of structural compounds from wood depends not only on temperature, time and pH (hydronium ions), but also on the wood particle size. Grinding increases the accessible surface area and decreases the crystallinity and polymerisation degree (Palmowski and Muller 1999) of the wood components. Mechanical pulping is a way of grinding where fibre layers (ML, P or S) are peeled off and expose the S2 cell wall. This facilitates the non-cellulosic polysaccharides extracted from secondary cell wall to water.

Extraction of structural compounds from wood is also a mass transfer process, where molecules and ions diffuse out from the wood, moving from regions of relatively high concentration into regions of lower concentration. The rate of mass transfer is dependent mainly on the concentration difference and the interfacial area, and also temperature. Cutting or grinding the wood to small particle size increases the interfacial area and increases the mass transfer rate. Temperature and pressure increase the rate of molecular movement, thereby increasing the rate of diffusion.

1.6 Extraction of galactoglucomannans from spruce TMP and wood

1.6.1 Isolation of galactoglucomannans from TMP water

Methods based on ultrafiltration have been developed for isolation of polymeric GGMs from TMP waters (Persson et al., 2010; Persson and Jönsson 2010) combined with precipitation in ethanol (Willför et al., 2003; Xu et al., 2007) (Figure 10). A yield of 5 mg/g of pulp was achieved in pilot scale tests, with GGM purity of 95% and average Mw up to 59 kDa.

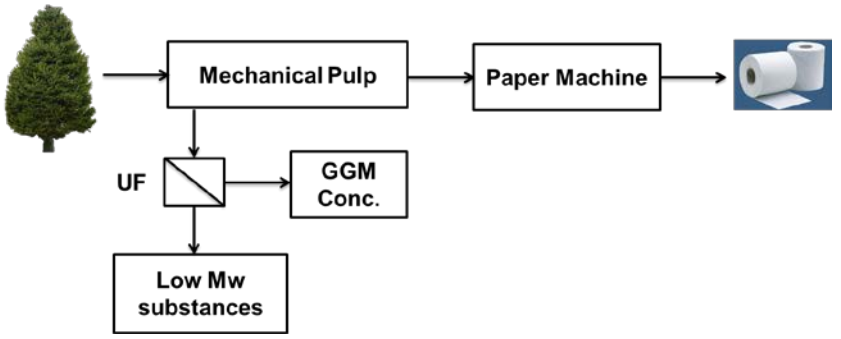


Figure 10. Isolation of GGMs from TMP water.

1.6.2 Extraction of GGMs from spruce wood

Water extraction at lower temperature

Spruce wood meal was extracted at 90°C for up to 12 hours (wood:water ratio 1:100, Örså et al., 1997). After the extraction, the total non-cellulosic carbohydrates, mostly GGM-derived glucose and mannose, released from spruce wood amounted to only about 14 mg/g of wood. The influence of pH of suspension, and salt additions (NaCl and $CaCl_2$) were also studied. The yield of non-cellulosic carbohydrates was found to be lower when the pH-level was 4.5 – 6.7.

Microwave-assisted extraction

The wood:water ratio was set to about 1:10 and diffusion rate was believed to be enhanced by microwave irradiation (Lundqvist et al., 2003). After

microwave heat treatment, the insoluble materials (mainly cellulose and lignin) were removed by filtration. The filtrates contained mostly water-soluble oligo- and polymeric GGMs. At 190°C and 5 min heat treatment, about 78% of the wood mannans (oligo- and polymeric) were extracted. The average molar mass of the extracted mannans was 3.8 kDa. When temperature and time were increased, the average Mw were decreased. Different conditions (%NaOH, temperature and residence time) were also evaluated.

Pressurised flow-through extraction

A flow-through extractor was used by Leppänen et al. (2011) (Figure 11).

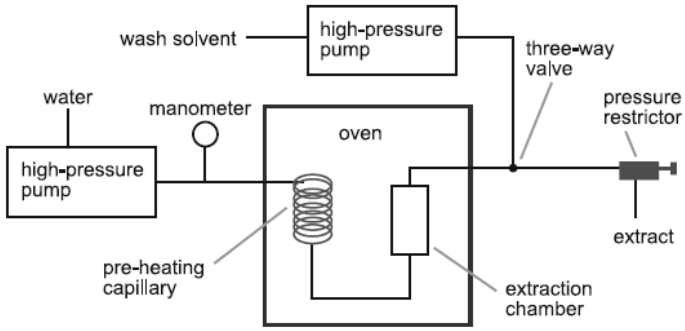


Figure 11. Pressurised hot-water flow-through extraction set-up (Leppänen et al., 2011).

Spruce saw meal was extracted in an extraction chamber (wood:water ratio in the chamber was 1:15) by steady flow-through of water. The pressure was 150 bar and different temperatures were evaluated.

Large amounts of water were used before the collection of extract. However, an extract with an average molar mass of 31 kDa was obtained at 170°C, but the yield of total non-cellulosic carbohydrates was only about 70 mg/g of wood. Higher yields of non-cellulosic carbohydrates were achieved by increasing the temperature, but the average molar mass of the extracts was decreased.

Pressurised hot-water extraction by ASE

Pressurised hot-water extraction by Accelerated Solvent Extractors (ASE) has

been used for extraction of softwoods in our laboratory, but has been used also for hardwoods (Tunc et al., 2008a and 2008b; Jara 2010).

ASE (Figure 12) is an efficient static batch extraction technique with good repeatability at extraction temperatures up to 200°C. ASE also enables sequential extractions of the same sample, for example, starting with organic solvents to remove lipophilic and semi-polar extractives, and then continuing with hot water to extract mainly non-cellulosic carbohydrates, and finally with alkaline hot water to extract lignin.

Figure 12. Accelerated Solvent Extractor apparatus (ASE-300).

ASE has extraction cells which can be heated up to 200°C in an oven, a pump, bottles for extraction solvents and collection vials for extracts (Figure 13).

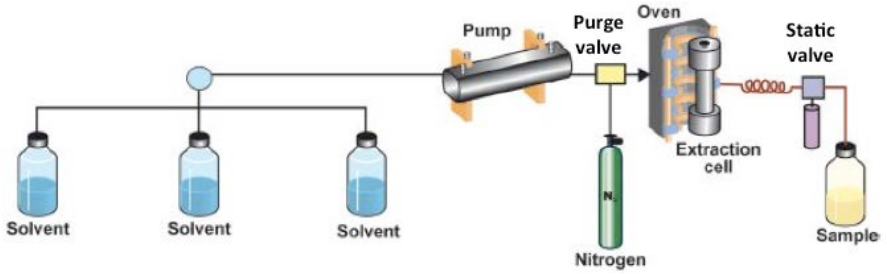


Figure 13. ASE scheme.

There are also a static valve and a purge valve which are used to control pressure and purging. Extraction cells of different sizes are available, with different volumes where up to about 20 g (weight is on dry basis) of wood can be extracted.

The extraction cell with contents is loaded into the oven after the temperature of the oven has reached the set-point. Then the pump starts pumping the

solvent into the cell until the cell is full (solid:water ratio is about 1:4), and the static valve is closed and the flow stopped. The cell is then heated for a fixed time to ensure that the sample reaches thermal equilibrium. During the heating, the static valve opens periodically to maintain the set-point pressure in the cell. After the extraction, the static valve is opened and extract is purged out into the collection vial with nitrogen. Fresh solvent is flushed through the cell (usually 50 to 100% of the cell volume) to purge and rinse the extraction cell content. The remaining solvent in the extraction cell is purged again with nitrogen gas.

Isolation of GGMs by ultrafiltration and precipitation in ethanol from spruce wood

Al Manasrah et al. (2012) and Pranovich et al. (2010) isolated GGMs from pressurised hot-water extracts of spruce wood by ultrafiltration and precipitation in ethanol, respectively.

Al Manasrah isolated GGMs from the extracts obtained by pressurised flow-through extraction. Three membranes were used. The highest GGM retention (88%), purity (63%) and recovery (70%) were achieved with the UC005 membrane (cut-off value 5 kDa) at a volume reduction (VR%) of 86%.

The extracts obtained from spruce wood with ASE at 170°C and different extraction times were precipitated in ethanol. GGM-units were the main components in the precipitates. The highest yield was achieved to about 72 mg/g of wood at 20 min. The average molar mass of the ethanol precipitates decreased along the extraction time from 21 kDa to 5 kDa.

1.7 P-factor

The P-factor has been commonly used in hot-water extraction and pre-hydrolysis studies (Sixta 2006, Tunc 2008a, Jara 2010). It is a measure of the extraction intensity obtained by combining the temperature and time with aid of the Arrhenius equation. Although it is practical in technical studies, its value is questionable in scientific studies on the process chemistry because chemistry can be different at different temperatures and pH even if the

P-factor is the same.

1.8 Objectives of the work

The main objective of this thesis work was to obtain better fundamental understanding of hot-water extraction of softwood. An improved knowledge in this regard could facilitate the development of technical processes based on structurally unchanged non-cellulosic polysaccharides obtained from wood.

Understanding the critical parameters influencing the extraction of polymeric GGMs in high yield was a specific objective. Well-defined spruce wood material was extracted using ASE. Both water extracts and residues were analysed in detail to get understanding of the chemistry at the molecular level. Furthermore, polymeric GGMs were isolated from water extracts by membrane filtration and precipitation in ethanol, and their stability in water was studied.

2. MATERIALS AND METHODS

2.1 Materials and chemicals

2.1.1 Spruce wood and TMP

Healthy and mature spruce trees felled in Houtskär, Southwest Finland in September 2006 and May 2008 were used for the extractions. Knot-free stem discs were sawn out, and after removing bark from the discs, sticks were cut out from the sapwood and heartwood parts of the discs. The sticks were stored at -24°C.

The sticks from the tree felled in 2006 were used for the studies of water extraction of spruce chips and ground sapwood (I), pH effects on the extraction by addition of $NaHCO_3$ (II), pH control by phthalate buffers (III) and the influence of particle size on the extraction (IV). For the studies of sapwood and heartwood extractions (IV), purification and characterization of GGMs from wood extracts (V), sticks from the spruce tree felled in May 2008 were used.

Chips were manually cut out from the fresh sapwood with the dimensions of 20 mm long, 10 mm wide and 3 mm thick, and with a 45° cutting angle. The wood sticks from sapwood and heartwood were ground separately in a Wiley mill equipped with a 2-mm screen. The ground heartwood and a part of the sapwood were further ground with a 1-mm screen.

Another part of the ground sapwood, to be used for particle size distribution studies, was air-dried and further separated into four fractions with different particle sizes (<0.1, 0.25 – 0.5, 0.75 – 1 and 1.25 – 2 mm) using a kit of sieves and a Retac 3D vibrating sieve shaker (Retsch, Haan, Germany). All ground wood materials were stored at -24°C in sealed polyethylene bags in the dark until extraction.

Norway spruce thermomechanical pulp (TMP) was sampled after the second refiner in a Finnish pulp mill (UPM Kaipola). The dry content of the pulp

was about 45%.

2.1.2 Chemicals

Seven $NaHCO_3$ solutions were prepared in distilled water with concentrations of 2.5, 5, 12.5, 25, 50, 100 and 150 mM.

Four different phthalate buffer solutions, with pH 3.8, 4.0. 4.2 and 4.4, were prepared. The pH of the phthalate buffer solutions was adjusted by adding 0.1 M HCl or NaOH to 0.1 M potassium hydrogen phthalate.

2.2 Methods

2.2.1 Extraction of spruce wood

Pressurised hot-water extractions of spruce wood were performed with an ASE-200 or an ASE-300 apparatus (Dionex, Sunnyvale, CA, USA). Approximately 7 g (ASE-200) or 20 g (ASE-300) (all weights here and further are oven-dry weights) ground wood was weighed and extracted at different temperatures (up to 180°C) and time (up to 100 min) with 30 ml (ASE-200) or 80 ml (ASE-300) of chosen water solvent. The pressure in the extraction cell was 2000 psi (ca. 138 bar, ASE-200) and 1500 psi (ca. 100 bar, ASE-300). After the extraction, the extract solution was purged out with nitrogen and rinsed with 20 ml (ASE-200) or 50 ml (ASE-300) of corresponding extraction solvents. The volume and end-pH of the extract solutions were measured at room temperature shortly after the extraction. All water extracts were stored in closed test tubes at 4°C in the dark.

2.2.2 Purification of galactoglucomannans

Altogether about 4.5 l of water extract was prepared by multiple ASE extractions (ASE-300, 170°C, 20 min, 43 batches) from a total amount of about 1 kg spruce wood. The water extracts were centrifuged and the fine sediments were removed (mostly lignin and lignin-related substances, II). The volume and end-pH of the extract solutions were measured at room temperature shortly after the extraction.

Purification of GGMs by membrane filtration

The water extract was purified by membrane filtration using Jumbosep™ Centrifugal Devices (Pall Corporation, New York, US). The devices were designed originally for globular solute filtration of protein and virus solutions. The study aimed at isolating GGM extracts with different average molar masses. Modified polyethersulfone membranes with five pore sizes (300, 100, 30, 10 and 3 kDa based on globular solute filtration ability, low protein-binding) were used. The device was assembled as illustrated in Figure 14 after adding the water extracts into the sample reservoir.

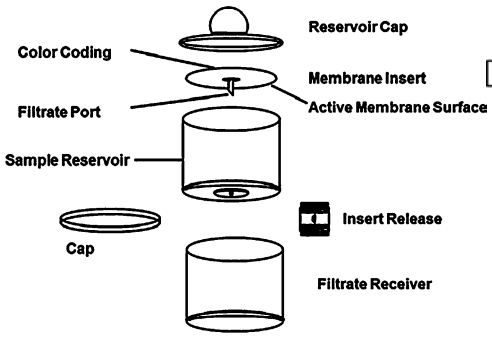


Figure 14. Set-up of Jumbosep™ Centrifugal Devices.

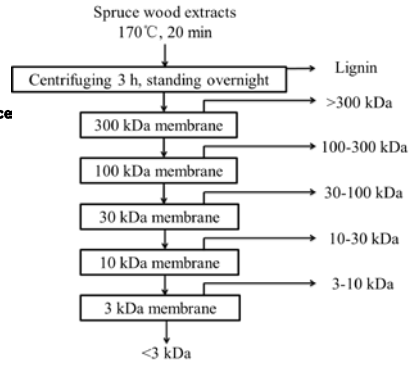


Figure 15. Filtration scheme

The assembled device with contents was then centrifuged at about 1575g for 24 hours. The filtration was started with the largest pore size membrane (300 kDa). After each filtration, the extract in the reservoir and the filtrate in the receiver were both transferred to test tubes and stored at 4°C in the dark. The filtrate passing each membrane filter, first the 300 kDa membrane, was used for the next filtration with the next smaller size membrane (100 kDa), and so forth, as outlined in Figure 15.

Separation of GGMs by precipitation in ethanol

The aim was to find the optimal precipitation conditions for isolation of high-molar-mass GGMs from water extracts (V). Centrifuged water extract with a reduced content of lignin was used for precipitation of GGMs. High-molar-mass non-cellulosic carbohydrates were precipitated by adding the water extracts to different volumes of ethanol (99.5 % purity) to obtain different ethanol-water ratios. The total volume of the ethanol-water solution

was 400 ml.

After precipitation, the suspensions were left to stand overnight. The supernatants were decanted and the precipitates filtered and washed with ethanol, acetone and MTBE, and finally vacuum-dried. The dried precipitates were white powders. The precipitates were stored at 4°C in the dark. About 20 mg of each precipitate was weighed and dissolved in 10 ml distilled water. Aliquots of sample solutions were taken for different analyses.

2.2.3 Isolation and treatment of galactoglucomannans in stability study

About 15 g of ground spruce wood with a particle size smaller than 1 mm was extracted in an ASE-300 apparatus with water at 170°C for 20 min. Polysaccharides were precipitated by addition of water extracts to ethanol to obtain a water:ethanol ratio of 15:85 by volume. The precipitated white powder was separated by filtration on a glass fibre filter, washed with ethanol, acetone and MTBE, and finally vacuum-dried.

Analysis of the powder by acid methanolysis and GC showed that the GGM sugars (Gal + Glc + Man) amounted to 82% of the sugars, the rest being mainly xylan sugars (Xyl + Ara + 4-O-MeGlcA, 11%) and pectin sugars (GalA + Rha, 7%). The molar mass determined by HPLC-MALLS was above 4 kDa, with an average Mw of 8 kDa.

In the stability studies, about 0.4 g isolated GGM powder was added into ASE-300 cells. One of the phthalate buffer solutions with starting pH of 3.8, 4.0 or 4.2 was added, and the cell content was treated at 170°C for various times, up to 60 min. The final volume of the extract solutions was about 50 ml.

2.2.4 Characterisation methods

Total dissolved solids (TDS)

Total dissolved solids (TDS) were determined gravimetrically after freeze-drying to a constant weight from 2-ml aliquots of the extract solution.

Total non-cellulosic carbohydrates

Total non-cellulosic carbohydrates were determined by GC after acid methanolysis that cleaves non-cellulosic polysaccharides to monomer sugars by forming methyl glycosides (Sundberg et al., 1996). This determination collects mainly sugar units from non-cellulosic carbohydrates, but also from mono- and oligosaccharides.

Monosaccharides

Monosaccharides in water extracts were determined by GC of an aliquot of the extract solutions after freeze-drying and silylation of the dry residue.

Total non-cellulosic carbohydrates in wood residues after extraction

The residual non-cellulosic carbohydrates in extracted ground wood samples were analysed by GC after freeze-drying and acid methanolysis, as described above.

Acetic acid

Free acetic acid in the extract solutions, released during ASE extraction, was analysed by HPLC with a Synergi Hydro-RP 80R HPLC Column (250 mm × 4.6 mm, 4 mm, Phenomenex®, Torrance, CA, USA) and an RI detector (Shimadzu, Tokyo, Japan). The pH of the extract solution was adjusted to 2.7 – 2.9 with 30% *ortho*-phosphoric acid. The eluent contained 20 mM KH_2PO_4 in pure water and pH was also adjusted to 2.7 – 2.9 with *ortho*-phosphoric acid. The eluent was filtered with a 0.1-mm filter (Anodisc 47, Whatman International, Maidstone, UK). The injection volume was 100 µl.

Acetyl groups in dissolved hemicelluloses were hydrolysed by alkaline treatment of extract solutions at 70°C for 3 h, after adjusting the pH to 12 with a 1 M sodium hydroxide solution. After treatment, the pH was adjusted to 2.7 – 2.9 with *ortho*-phosphoric acid. Hydrolysed acetic acid was determined by HPLC as described above.

Lignin and lignin-related substances (LLRS)

Two UV-spectroscopy methods were used to determine the content of lignin and lignin-related substances (LLRS) in extracts.

LLRS in water extracts obtained from plain-water extraction (I) and extraction with addition of $NaHCO_3$ (II) were measured by UV absorption at the wavelength 280 nm in sodium acetate buffer after acetyl bromide treatment. Aliquots of total water extracts, including precipitated lignin, were freeze-dried and then dissolved in acetic acid (AcOH) with acetyl bromide (AcBr) (AcBr:AcOH 1:3 v/v) at 70ºC. After the dissolution, the solution was diluted to 100 ml with AcOH and measured by UV-spectrometry. The extinction coefficient used for lignin determination was 43 cm•mg/l (Iiyama and Wallis 1988).

In studies III, IV and V, LLRS in water extracts was measured by UV absorption at the wavelength 280 nm. Aliquots of water extracts were measured directly by UV after different dilution times until an absorbance value of 0.3 – 0.7/cm was reached. The extinction coefficient used for lignin determination was 56 cm•mg/l, which was obtained from milled wood lignin in our laboratory.

Average molar mass (Mw)

Weight-average molar masses (Mw) were determined by high-performance size-exclusion chromatography (HPSEC) equipped with a multiangle-laser-light-scattering (MALLS) detector (miniDAWN, Wyatt Technology, Santa Barbara, CA, USA) and an RI detector (RID) (Shimadzu, Tokyo, Japan). The system consisted of a guard column (Ulrahydrogel 6 mm × 40 mm, Waters, Milford, MA, USA) and two columns (2 × Ultrahydrogel™ linear 7.8 mm × 300 mm, Waters, Milford, MA, USA) connected in series. The water extracts were filtered through a 0.22-mm nylon syringe filter before injection. Eluent: 0.1 M sodium nitrate solution; flow rate: 0.5 ml/min; injection volume: 100 µl. Astra software (Wyatt Technology) was used to calculate average molar masses. A dn/dc value of 0.15 ml/g for GGMs was taken for the calculations (Michielsen 1999).

2.2.5 Structural characterisation of galactoglucomannans

Hydrophobic and semi-polar substances, including lignin and other aromatic substances, were removed from the hot-water extract of spruce wood by adsorption on a column packed with XAD-7 resin (Pranovich et al., 2005). A rather pure GGM-rich white powder was obtained by precipitation of the

lignin-free extract in ethanol:water (85:15 v/v) as described above (chapter 2.2.3).

The purified GGM-rich powder was dissolved in D_2O, and 2, 2-dimethyl-2-silapentane-5-sulfonic acid (DSS) was added as internal standard. Quantitative ^{13}C NMR spectra were recorded with a Bruker Avance NMR spectrometer at 150.90 MHz, with the probe temperature at 323 K. An inverse-gated pulse sequence, which was 18-s pulse delay (D1) was used. About 17000 scans were accumulated.

2.2.6 Microscopy of ground wood particles

Optical microscopy of ground wood particles (never-dried) was made using a Leica Wild MZ8 stereo microscope interfaced with a digital camera.

3. RESULTS AND DISCUSSION

3.1 Hot-water extraction of spruce wood

Well-defined spruce chips and ground sapwood with different particle sizes were extracted with plain, pure water at various temperatures and times (I, IV). The extracts and wood residues were analysed in detail. Ground sapwood was also compared to ground heartwood and TMP fibres (IV), but only small differences were found and these results are not presented and discussed in this summary.

3.1.1 Influence of wood particle size and wood tissue

Total Dissolved Solids (TDS)

TDS, i.e. the total extraction yield, increased with the temperature and extraction time (Figure 16 – 17). However, a maximum was reached after 50 – 100 min time.

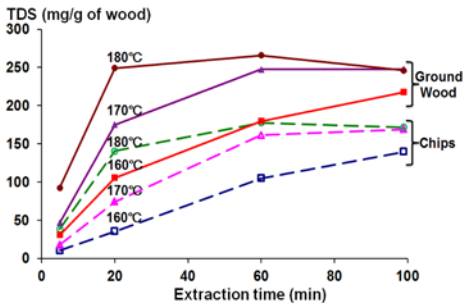


Figure 16. Total dissolved solids extracted from ground sapwood (< 1 mm) and chips at 160 – 180°C.

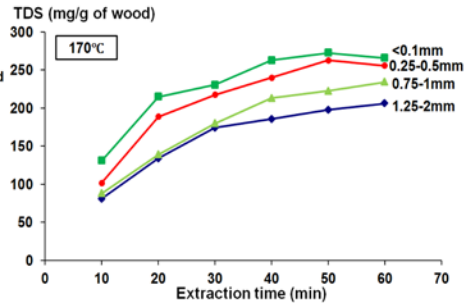
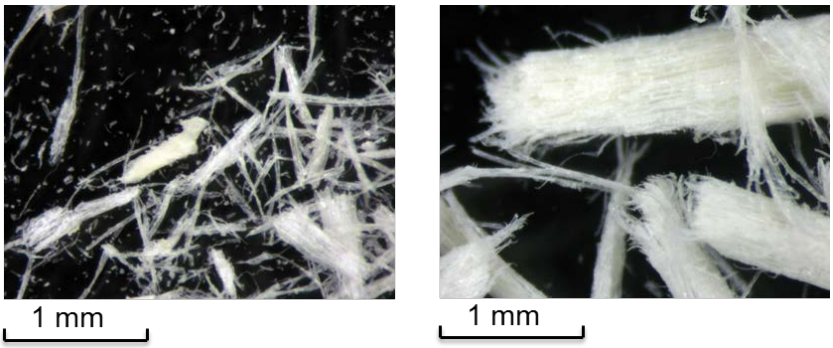


Figure 17. Total dissolved solids extracted from ground sapwood of different particle sizes at 170°C.

The yields from chips were only 40 – 60% of those from ground wood (Figure 15). The difference was the largest at short extraction times.

Wood ground to a very small particle size gives a higher extraction yield, especially at short extraction times (Figure 17). Wood meal (< 0.1 mm) gave 20 – 30% higher yields than coarse ground wood (1.5 – 2 mm). The higher

yield from small wood particles results from the larger surface area exposed and the higher degree of fibrillation obtained by extensive grinding (Figure 18). The diffusion of wood components is facilitated. It should, however, be considered that grinding consumes much energy. Moreover, the fibres will be damaged, which decreases the pulp and paper strength properties.



0.25 – 0.5 mm 0.75 – 1.0 mm

Figure 18. Microscopy images of two ground spruce sapwood fractions.

The water solutions were clear immediately after extraction, when still hot, but a brownish fine precipitate was formed when the solutions were cooled. This was the case for all extracts in the hot-water extraction studies. Analysis of the precipitates showed that lignin was the main component, amounting to about 60% of the precipitates, while 22% was carbohydrates (III).

End-pH

During hot-water extraction pH gradually decreases, down to levels of about 3.5 (Figure 19 – 20).

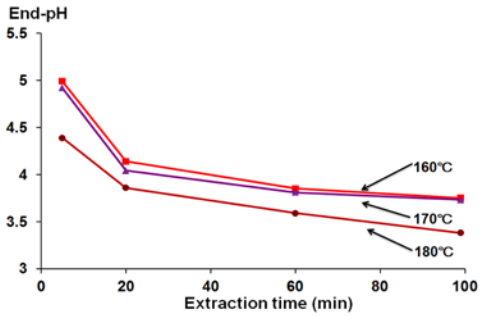


Figure 19. End pH of the water extract solutions from ground sapwood (< 1 mm) at different temperatures, measured at room temperature.

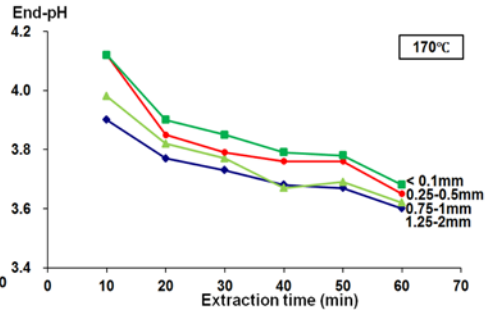


Figure 20. End-pH of the water extract solutions from ground sapwood of different particle sizes at 170°C, measured at room temperature.

The decrease is larger at higher temperatures. Finely ground wood meal exhibited a higher initial pH level than coarse ground wood and this difference was maintained throughout the extraction. The pH drop results from release of acids, mainly by acid hydrolysis of acetyl groups, as discussed below. Formic acid has also been found in hot-water extracts (Lehto and Alén 2012). Uronic acid units in pectins and xylans may also be involved (Casebier et al., 1969; Lai 2001).

Non-cellulosic carbohydrates

With time, up to 170 – 190 mg/g of carbohydrates are extracted from ground wood, comprising 60 – 80% of the extracted wood materials (Figure 21) (I).

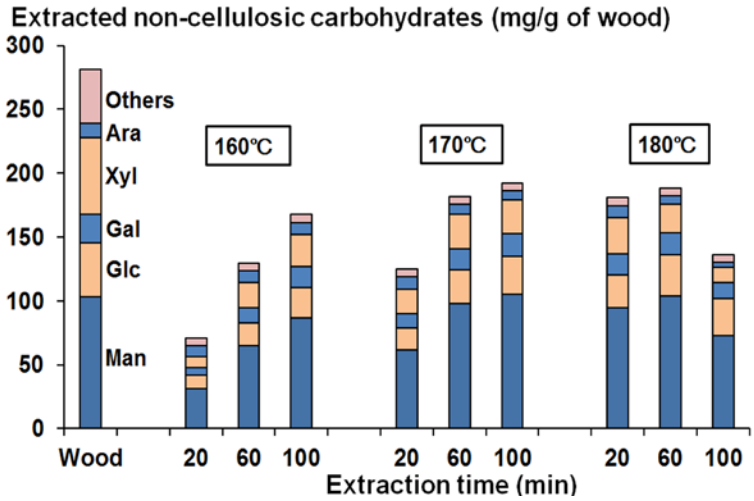


Figure 21. Amount and sugar composition of carbohydrates extracted from ground sapwood (< 1 mm) at different temperatures.

The carbohydrate yields dropped clearly for 100 min extraction time at 180°C. The decrease was most extensive for arabinose and xylose, probably due to their acidic degradation, primarily to furfural (Lai 2001). The yields of GGM-derived hexose sugars also decreased slightly. The decreased yield of GGMs is probably related to hydrolysis of acetyl groups, leading to lower water solubility of GGMs.

The amounts of galactose, glucose and mannose units accounted for approximately 75% of the total carbohydrates, indicating a preferable extraction of GGMs. In spruce wood, GGMs account for only 59% of the non-cellulosic carbohydrates. The extraction yield for GGMs was at the level of 80 – 90% (based on the amount in wood) between 170°C and 180°C. However, the extraction yield for xylan was only approximately 30%.

The highest yield of poly- and oligosaccharides was obtained from the finest particle size fraction (< 0.1 mm) after 40 min extraction, amounting to about 130 mg/g (Figure 22). This comprises about 50% of the corresponding TDS. Coarser fractions gave lower yields of total poly- and oligosaccharides. For all fractions, the yield of total poly- and oligosaccharides levelled off after 40 min extraction.

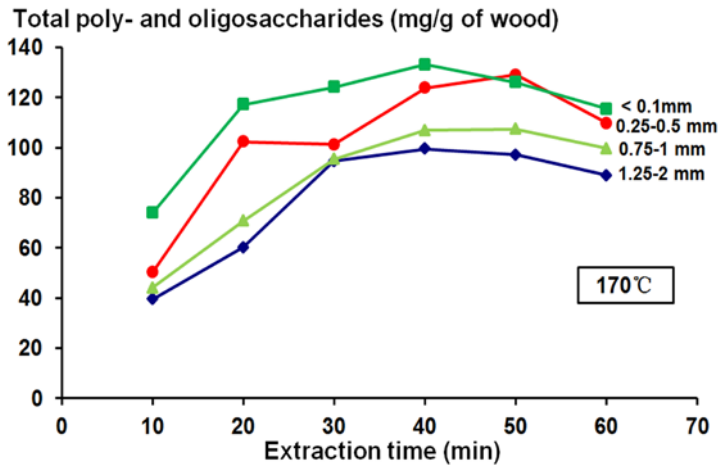


Figure 22. Amount of poly- and oligomeric carbohydrates as anhydro sugars extracted from ground sapwood of different particle sizes at 170°C.

With an extraction time of 10 – 20 min, the yields of poly- and oligosaccharides from the coarsest fraction were only half of the yields from the finest fraction. However, after longer extraction time, the differences became smaller. Acid-catalysed degradation of pentoses to furfural during extractions, especially during the extractions of finer particles, could be an explanation to this decrease, but the main reason most probably, is hydrolytic cleavage of poly- and oligosaccharides to monosaccharides that started to be extensive after 20 min, and was more extensive for finer fractions (Figure 24).

Monosaccharides

During hot-water extraction, the glycosidic bonds in hemicelluloses and pectins are partly hydrolysed (Casebier et al., 1969; Lai 2001; Karlsson et al., 2006; Yoon et al., 2008; Grénman et al., 2011; Leppänen et al., 2011), and considerable amounts of monosaccharides are formed, especially at elevated temperatures and extended extraction times (Figure 23). All extracts had very similar monosaccharide composition.

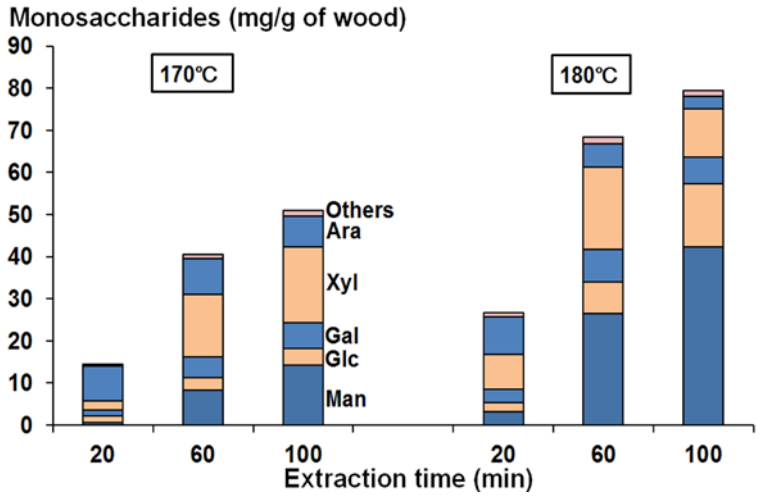


Figure 23. Amounts and composition of monosaccharides in water extracts, obtained from spruce ground sapwood (< 1 mm) at different temperatures.

Arabinose and xylose units were preferentially hydrolysed from xylans and arabinogalactans, but GGM-derived monosaccharides were also abundant in extracts at the most severe conditions.

The amounts of monosaccharides in the extracts increased clearly with decreasing particle size (Figure 24). The largest amounts of monosaccharides were obtained from the finest fractions, < 0.1 mm and 0.25 – 0.5 mm, after 60 min extraction.

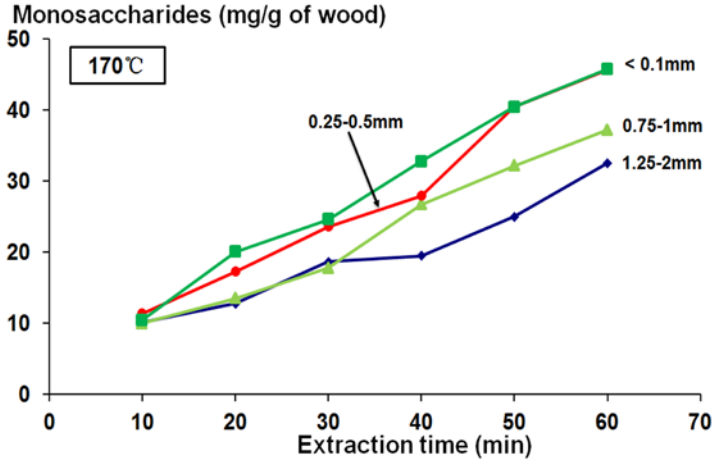


Figure 24. Amounts of monosaccharides in water extracts extracted from

ground sapwood of different particle sizes at 170°C.

At a giving extraction time, the composition of monosaccharides was the same for all particle size fractions.

Acetic acid

The amount of free acetic acid released from GGMs during hot-water extraction increased with temperature and time. The acetylation degree of GGMs was calculated relative to the amount of mannose units in the extracts, assuming that all acetyl groups are bound to the mannose units, as documented to be the case for GGMs released from thermomechanical pulp (TMP) (Hannuksela and Hervé du Penhoat 2004). The molar Ac/Man ratio is approximately 0.50 in spruce wood. A high degree of acetylation is necessary for good water solubility, and high extraction yield of high-molar-mass GGMs.

The extracted GGMs still had an Ac/Man ratio above 0.4 at the milder extraction conditions (Figure 25). However, at the most severe conditions, 180°C and 100 min, essentially all acetyl groups were hydrolysed from the dissolved GGMs.

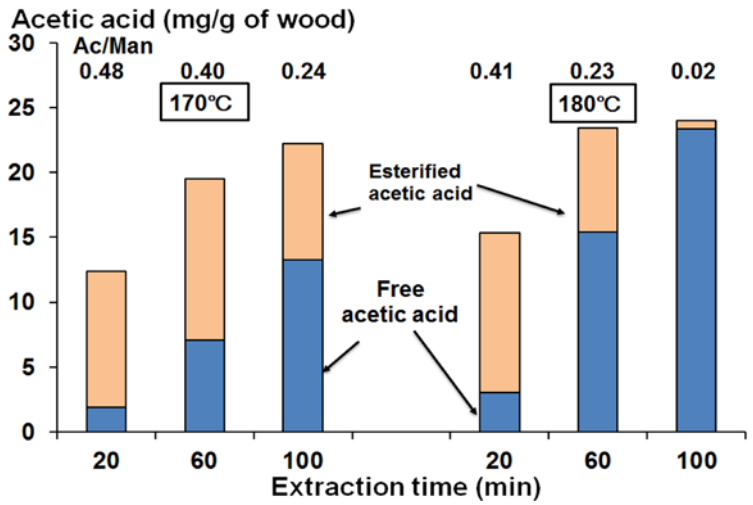


Figure 25. Amount of free acetic acid released from ground spruce sapwood (< 1 mm) during ASE extraction at different temperatures and in the form of

acetyl groups in the extracts.

The amount of free acetic acid released during the extraction of ground wood with different particle sizes increased almost linearly with the extraction time (Figure 26).

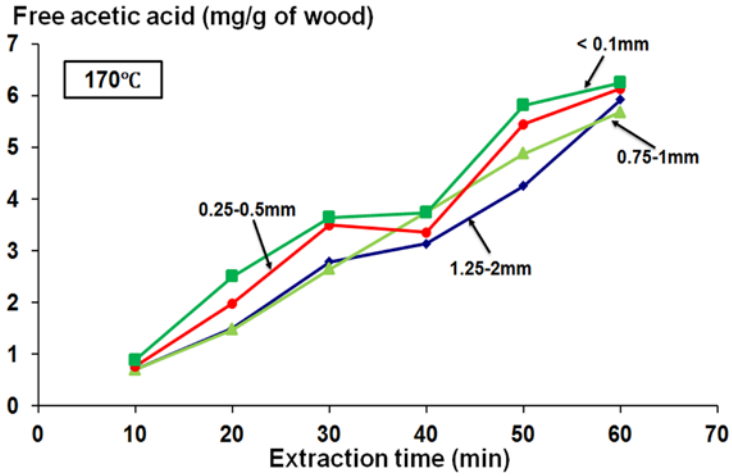


Figure 26. Acetic acid released during the hot-water extraction from ground spruce sapwood of different particle sizes at 170°C.

The finest fraction gave the largest amounts of acetic acid, i.e., the highest deacetylation of GGMs during the extraction. However, the wood particle size influenced deacetylation rate only slightly.

Average molar mass (Mw)

All water extracts were analysed by HPSEC-MALLS to determine the molar mass distribution and average molar mass (Mw) of dissolved GGMs and other carbohydrates.

High-molar-mass components were extracted at milder conditions (Figure 27). Extraction at 160°C for 5 min gave the highest Mw, being 35 kDa. Products with the lowest Mw were found at 180°C, 100 min, i.e., at the most severe conditions applied, where the hydrolysis is most extensive.

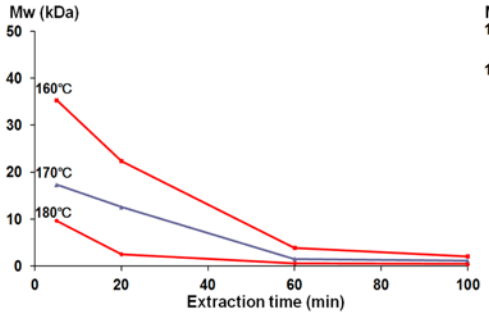


Figure 27. Average molar masses of non-cellulosic carbohydrates extracted from ground sapwood (< 1 mm) at different temperatures.

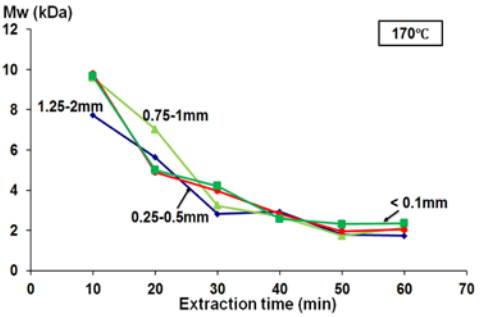


Figure 28. Average Mw of extracted non-cellulosic carbohydrates from ground sapwood of different particle sizes at 170°C.

At 170°C, high-molar-mass components were extracted at shorter extraction times from ground wood with different particle sizes (Figure 28). However, the decrease in Mw along the extraction was independent of the particle size.

Lignin and lignin-related substances (LLRS)

LLRS in the extracts released during the extraction of ground sapwood and chips was determined by UV-absorption measurement. More lignin was extracted at higher temperatures and longer extraction times (Figure 29). Again, ground wood gave higher yields than chips.

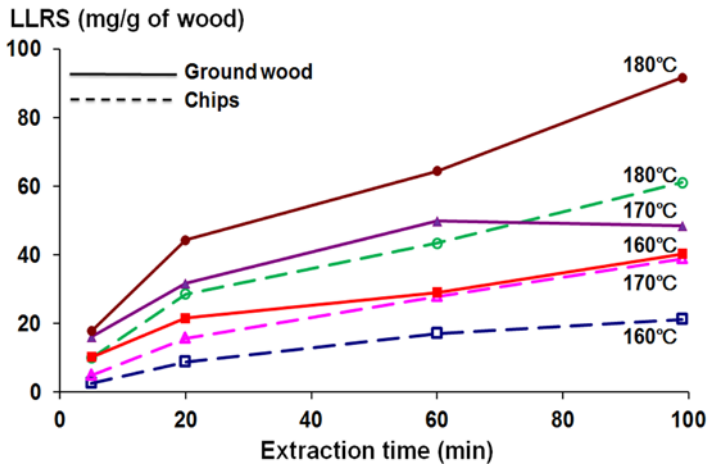


Figure 29. Amounts of lignin in water extracts obtained from ground spruce wood (< 1 mm) and chips at different temperatures.

Extracts obtained at severe conditions were darker, and extracts from ground wood were darker than those from chips. According to these data, 15 – 20% of the extracted wood material was lignin, with clearly higher proportions at the most severe conditions where approximately 30% of the lignin in wood was extracted. The reported yields (present and following) are approximate because the UV-extinction coefficient of lignin in the extracts is not accurately known and may vary with the extraction conditions. Lignin-related phenolics, lipophilic extractives and carbohydrate-derived compounds may also contribute to the absorption of UV-light at 280 nm, thus leading to overestimation of the lignin amounts.

Composition of wood residues

The high extraction yields for GGMs were verified by analysis of ground wood residues after extraction (Figure 30).

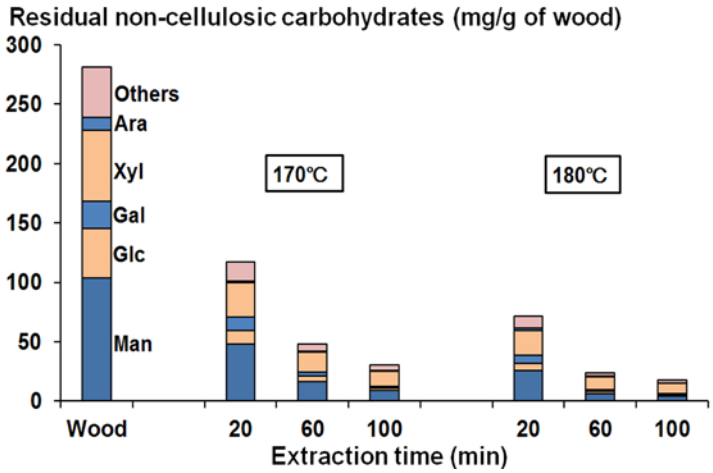


Figure 30. Amount and sugar composition of residual non-cellulosic carbohydrates in extracted ground sapwood (< 1 mm), calculated on original dry wood basis.

Very little galactose and mannose units remained in the wood after 60 min or longer extraction times at 170°C and 180°C. The amounts of xylose units also decreased, but to a lower extent. Relatively large amounts of glucose were found in the residues. Probably, most of this glucose originated from partial degradation of cellulose in the methanolysis procedure used for

analysis. Therefore, all these glucose amounts should not be assigned to GGMs. The glucose in GGMs was calculated based on the ratio of mannose to glucose (4:1) (Hannuksela and Hervé du Penhoat 2004). The surplus of glucose was considered to be due to cellulose and was not included in Figure 30.

Concluding remarks

The size of ground wood particles affects the hot-water extraction, especially in the initial stage of extraction, with the strongest effects on the yields of TDS and non-cellulosic carbohydrates. The degradation reactions of polysaccharides, both depolymerisation and deacetylation, and further degradation of monosaccharides become dominating after 40 min at 170°C.

Galactoglucomannans, which still contain a major part of their acetyl groups, can be extracted from ground spruce wood by pressurised hot water in yields of 80 – 90%, corresponding to 13 – 15% on dry wood basis. Such high GGM yields can be obtained by batch extraction of ground wood with particle size smaller than 1 mm at temperatures of 170 – 180°C with an extraction of 60 min. Higher temperatures and longer extraction times will lead to lower pH levels, causing deacetylation of GGMs, and consequently, the solubility of GGMs and yield will decrease. In total water extracts, approximately 55% are GGMs, including GGM-derived oligosaccharides and monomers. Other compound groups in the extracts are xylans, arabinogalactans, lignin and acetic acid. Pectic polysaccharides, present in the primary cell walls, are also partially extracted. During the extraction, GGMs are partly hydrolysed, giving some GGM oligo- and monosaccharides. Xylans and arabinogalactans are hydrolysed faster and to a higher extent than GGMs.

Extraction of non-cellulosic carbohydrates is limited mainly by the diffusion in the fibre wall, and for coarse wood shives also by the mass transfer in the wood matrix. Monosaccharides and acetic acid, on the other hand, will diffuse out from the fibre wall much faster than other non-cellulosic polysaccharides. Furthermore, grinding increases both the number and the size of pores in the cell wall. This enhances the swelling of the wood fibre in water and facilitates the larger molecules move out from the cell wall. To obtain a high yield of GGMs with a reasonably high molar mass, the pH

profile is also a key factor, which should be optimised to minimise the hydrolysis of acetyl groups and the hydrolytic cleavage of GGM chains.

3.1.2 Extraction with water at different pH levels

The pH is a key factor for extraction of high-molar-mass GGMs in high yield. Extractions with addition of different amounts of $NaHCO_3$ and phthalate buffers were conducted to find out how pH affects the extraction especially of GGMs.

TDS and end-pH

The extraction yields and pH-values of water solutions before and after extraction of ground spruce wood are given in Table 2 and 3.

Table 2. pH level of $NaHCO_3$ solutions and total dissolved solids (TDS) in extract solutions from spruce ground sapwood at 170°C.

$NaHCO_3$ concentration (mM)	Starting pH	170°C, 60 min		170°C, 100 min	
		End pH	TDS (mg/g of wood)	End pH	TDS (mg/g of wood)
0	5.5	3.8	248	3.7	246
2.5	7.9	3.9	254	3.7	247
5	8.1	3.9	233	3.8	270
12.5	8.2	4.5	156	4.4	230
25	8.2	4.7	138	4.3	180
50	8.2	5.2	141	4.7	176
100	8.2	6.5	141	6.4	147
150	8.3	7.4	143	7.3	165

The extraction profiles with different $NaHCO_3$ concentrations were very similar after 100 min and 60 min. The highest yield of TDS after 60 min extraction, approximately 250 mg/g, was obtained with 2.5 mM $NaHCO_3$ solution. Plain water gave a slightly lower yield. Extraction with 5 mM $NaHCO_3$ still improved the results, but beyond this concentration the yields decreased.

Table 3. pH of phthalate solutions and total dissolved solids (TDS) in the extract solutions obtained from ground spruce sapwood at 170°C.

Starting pH	Extraction time (min)	End pH	*TDS (mg/g of wood)
Without buffer	20	3.89	148
	60	3.69	254
	100	3.50	253
3.80	20	3.79	149
	60	3.74	224
	100	3.69	240
4.00	20	3.96	132
	60	3.84	223
	100	3.75	225
4.20	20	4.16	66
	60	3.99	187
	100	3.86	229
4.40	20	4.28	63
	60	4.13	159
	100	3.96	222

* Amount of phthalate was omitted from TDS.

Phthalate buffer solutions with original pH of 3.8 and 4.0 gave slightly lower extraction yields than plain water. The yields with buffer solutions at pH 4.2 and 4.4 were clearly lower, especially after 20 min extraction time. After 100 min extraction with plain water, the pH had dropped to 3.5, while for the phthalate solutions pH decreased only slightly during the extractions.

Non-cellulosic carbohydrates

Higher $NaHCO_3$ concentrations giving higher pH levels, gave lower carbohydrate yields (Figure 31).

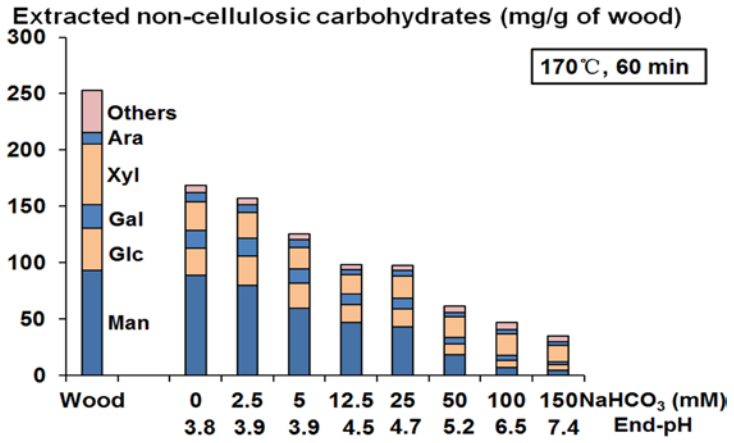


Figure 31. Amount and sugar composition of total non-cellulosic

carbohydrates (as anhydro-sugars) extracted from ground spruce sapwood at 170°C with $NaHCO_3$ solutions.

The decrease in yield was especially pronounced for the GGMs and GGM-derived carbohydrates. This can be interpreted as a result of alkaline hydrolysis of acetyl groups at higher pH levels, leading to lower solubility of GGMs in water. However, higher concentrations of $NaHCO_3$ did not affect the extraction of xylans very much. Approximately 40% of xylans and xylan-derived products (based on the xylans in wood) were extracted. The xylans extracted at higher $NaHCO_3$ concentrations contained more 4-*O*-methylglucuronic acid units, as observed in an increasing 4-*O*-MeGlcA:Xyl ratio.

Approximately 80% of the extracted total non-cellulosic carbohydrates (2.5 mM and 5 mM $NaHCO_3$, 170°C, 60 min) comprised galactose, glucose and mannose units, indicating a preferable extraction of GGMs and GGM-derived products.

With phthalate buffer solutions, higher yields were obtained at lower pH-levels (Figure 32). Compared to plain water, the buffer enhanced the extraction of non-cellulosic carbohydrates. The total yield of with pH 3.8 solution, amounted to about 155 mg/g of wood after 60 min extraction time, comprising 70% of the total dissolved solids. The yield decreased slightly after 100 min extraction mainly because the pentose sugars, arabinose and xylose, were degraded.

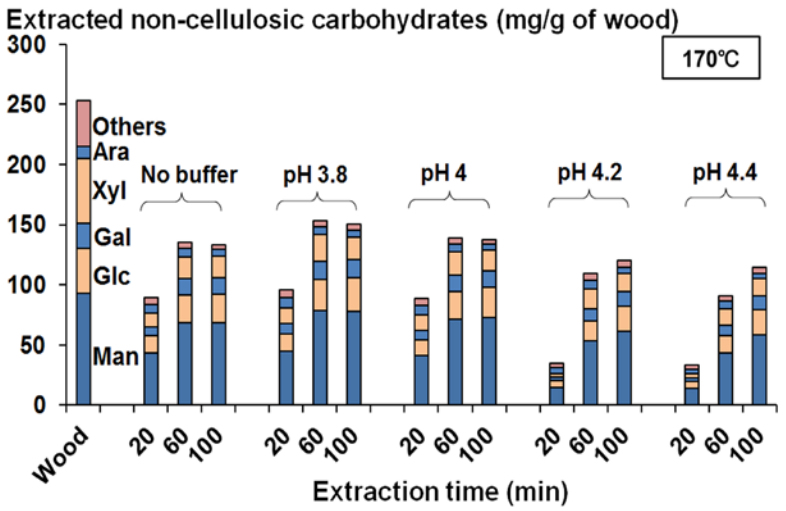


Figure 32. Total carbohydrates extracted from ground spruce sapwood with different phthalate buffer solutions at 170°C, compared to the content in original sapwood.

The phthalate buffer solutions with pH of 4.2 and 4.4 gave lower total yields of carbohydrates, mainly because of less efficient extraction of GGMs. At these pH levels deacetylation occurs, leading to a lower solubility in water of GGMs. About 70 – 80% of the carbohydrates extracted from wood with phthalate buffer solutions comprised galactose, glucose, and mannose units, implying preferable extraction of GGMs relative to xylans.

Monosaccharides

At low $NaHCO_3$ concentrations, monosaccharides were still formed in substantial amounts (Figure 33).

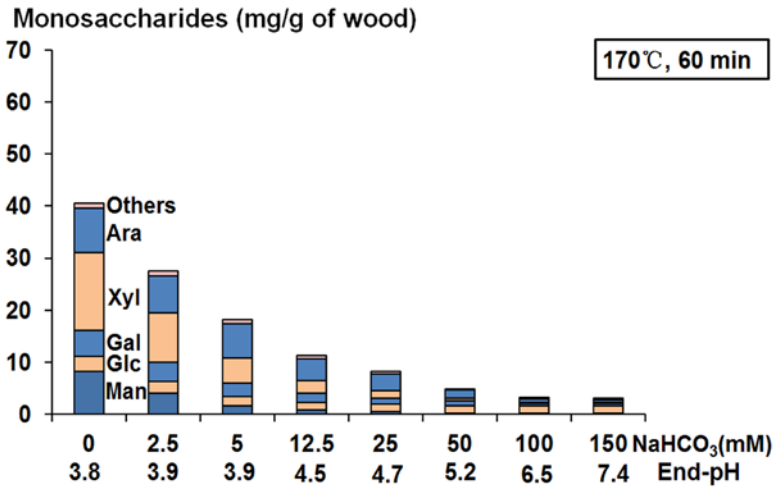


Figure 33. Amounts and composition of monosaccharides in NaHCO3 extracts from ground spruce wood obtained at 170°C.

Arabinose and xylose units in xylans were preferentially split off. However, at higher $NaHCO_3$ concentrations the hydrolytic cleavage of these pentoses decreased. The hexoses in GGMs were split off to a lower extent. The amounts of monomeric mannose and galactose sugars in extracts dropped gradually with increasing $NaHCO_3$ concentrations, verifying an inhibition of hydrolytic cleavage of GGMs.

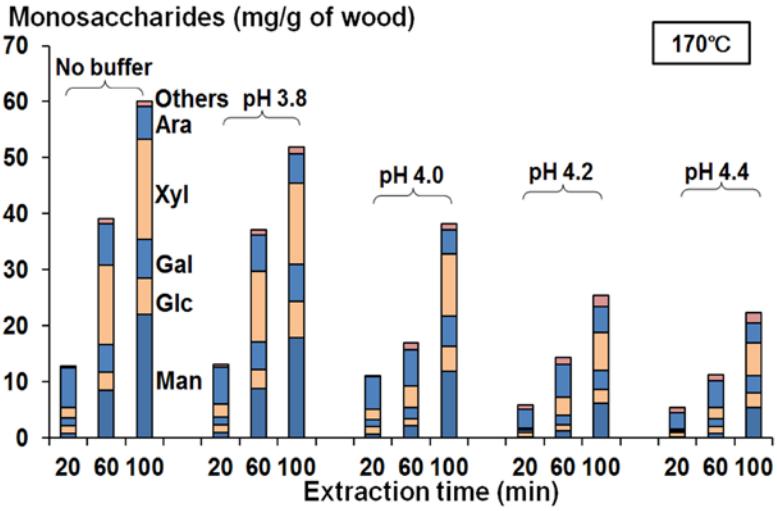


Figure 34. Monosaccharides in extracts from different phthalate buffer extractions at 170°C.

Monosaccharides were released also during extractions with phthalate buffer solutions, but to a lower extent than with plain, unbuffered water (Figure 34). At the pH levels 4.2 and 4.4, the amounts of released monosaccharides were only about one-third of that with plain water. Arabinose units were split off at an earlier stage of extraction than xylose and hexose units.

Acetic acid

The amount of released acetic acid in water extracts was the lowest at 2.5 and 5 mM $NaHCO_3$ concentrations because of inhibition of acid hydrolysis (Figure 35). However, at higher $NaHCO_3$ concentrations the deacetylation increased again due to alkaline hydrolysis. At the highest $NaHCO_3$ concentrations essentially all acetyl groups were split off. At low $NaHCO_3$ concentrations, the extracted GGMs still had Ac/Man ratios above 0.4. However, at elevated $NaHCO_3$ concentrations, the acetylation degree decreased.

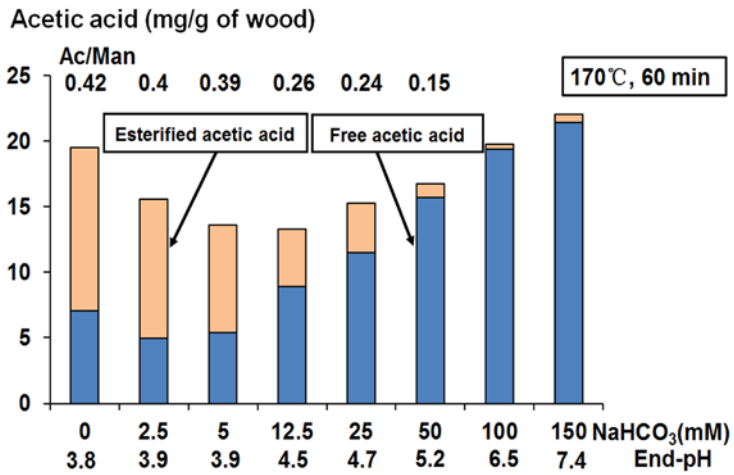


Figure 35. Acetic acid released during extraction and residual acetyl groups in extracted GGMs (esterified).

Hydrolysis of acetyl groups also occurred when phthalate buffer solutions were used for extraction. However, the deacetylation was much lower than with plain unbuffered water, especially after longer extraction time (Figure 36). It seems that the overall deacetylation was on the same level in the entire pH range of 3.8 – 4.4, without any distinct minimum. At higher pH levels

deacetylation will be very fast, thus decreasing the extraction of GGMs (II).

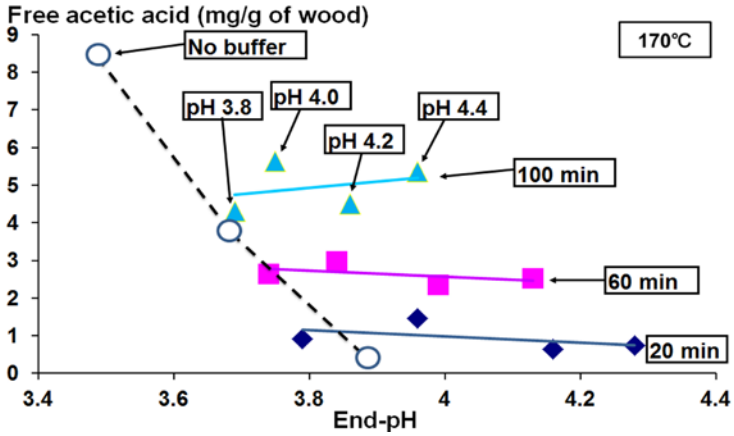


Figure 36. Acetic acid released from ground spruce wood during extraction with phthalate buffer solutions and plain, unbuffered water at 170°C.

The GGMs extracted with plain water still had an Ac/Man ratio above 0.4 after 20 min. However, after a longer extraction time, essentially all acetyl groups were hydrolysed from the dissolved GGMs. The degree of acetylation dropped to 0.19 and 0.08 after 60 and 100 min. With phthalate solutions the calculated Ac/Man ratio remained at about 0.3 even after long extraction times. These results agree well with the determined amounts of released (free) acetic acid.

Molar mass

The Mw of the extracted carbohydrates clearly increased with 2.5 – 5 mM $NaHCO_3$ solutions compared to extraction with plain water (II). The molar mass distributions of some extracts are illustrated with the HP-SEC chromatograms in Figure 37.

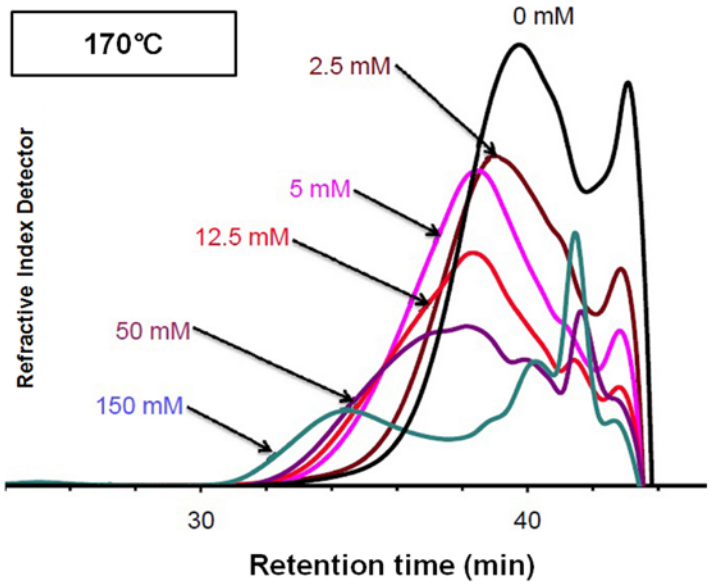


Figure 37. HPLC-SEC of extracts as function of $NaHCO_3$ concentration. 0 mM: plain water.

The peak in Figure 37 between 42 and 44 min, which decreases with increasing $NaHCO_3$ concentrations, is mainly due to monosaccharides formed from non-cellulosic carbohydrates during the extraction. As expected, the hydrolytic cleavage of polysaccharides chains during extraction is inhibited at higher pH values.

Molar-mass distributions of extracted non-cellulosic carbohydrates in phthalate solutions are presented here only for the 20-min extractions (Figure 38). Similar mass distributions were obtained for 60 and 100 min extraction times; all showing that higher pH levels give higher molar mass for the extracted carbohydrates.

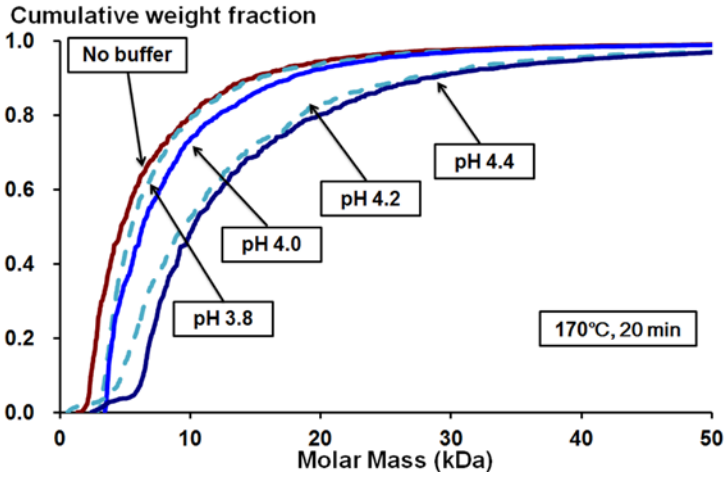


Figure 38. Cumulative molar-mass distribution (by weight) of extracted GGMs and other carbohydrates. Extraction for 20 min at 170°C with different phthalate buffer solutions.

The highest average molar mass (14 kDa) was obtained at the highest pH (4.4) after 20 min; however, the lowest pH (3.8) resulted in much lower average molar mass (8.5 kDa) than higher pH (4.2 and 4.4), but it still gave a higher average molar mass than plain water (7.5 kDa). These results are in accordance with the results for released monosaccharides (Figure 34) and are logical since hydrolytic cleavage of glycosidic bonds is dependent on the hydrogen ion concentrations.

Lignin and lignin-related-substances (LLRS)

The highest yield of LLRS was obtained with the 2.5 mM $NaHCO_3$ solution (Figure 39). With higher $NaHCO_3$ concentrations, the LLRS yield decreased gradually. However, at concentrations of $NaHCO_3$ higher than 50 mM the proportion of LLRS in TDSs increased up to 30 – 70%. This is because the yield of carbohydrates was lower in TDS.

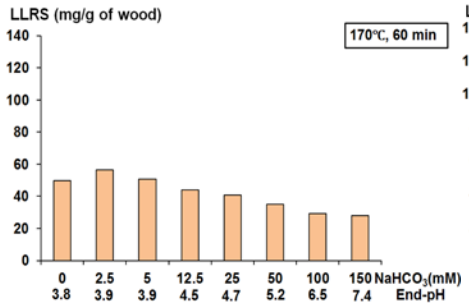


Figure 39. Amounts of lignin in $NaHCO_3$ extracts obtained from ground spruce sapwood at 170°C.

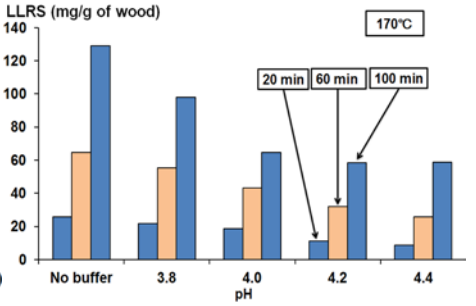


Figure 40. Lignin and lignin-related substances extracted from ground spruce sapwood with different phthalate buffer solutions at 170°C.

Less LLRS were extracted with the buffered water solutions than with plain water (Figure 40). By extraction with buffer solutions for 100 min, the amounts of extracted LLRS were 25 – 40% of TDS, corresponding to 21 – 35% of the total lignin in spruce wood (ca 280 mg/g). With 60 min extraction time the extracted LLRS amounts were clearly lower than with 100 min. For instance, with pH 4.2 buffer solution LLRS represented only 17% of TDS, which is 11% of the lignin in wood. The colour of all solutions was brownish, and the extract with plain hot-water after 100 min was the darkest.

Concluding remarks

Addition of $NaHCO_3$ in small amounts can enhance hot-water extraction of high-molar-mass non-cellulosic carbohydrates from wood by partially inhibiting the autohydrolysis of acetyl groups and glycosidic bonds. Extraction of spruce wood with 2.5 – 5 mM $NaHCO_3$, which gave an end-pH of about 4, inhibited autohydrolysis to some extent and yielded GGMs with higher molar mass. Higher $NaHCO_3$ concentrations, however, lead to extensive alkaline hydrolysis of the acetyl groups in the GGMs, and consequently to a low yield of GGMs. Xylans, arabinogalactans and lignins were extracted only to a slightly lower extent with hot bicarbonate solution having high $NaHCO_3$ concentrations.

The extraction of GGMs from spruce wood with addition of phthalate buffer confirmed results of our previous studies which had shown that the extraction

of spruce wood, with pressurised-hot-water is dependent on the pH in a narrow region around pH 4. This finding is important especially when galactoglucomannan is to be extracted in polymeric and/or oligomeric form. Extraction with phthalate solution (pH level around 4) gave as high a yield of GGMs extraction as with plain water, but considerably inhibited the hydrolysis of non-cellulosic carbohydrates and deacetylation of GGMs. Much less oligo- and monosaccharides, were found in the water extracts with addition of phthalate than with plain water.

3.2 Fractionation of hot-water extracts by filtration and precipitation

Filtration of GGMs was performed by filtration using a series five membranes with different pore sizes. The extracts used for filtration were centrifuged to remove most of the precipitated lignin.

The same extracts were also used for the precipitation in ethanol. Nine water:ethanol ratios were compared.

Mass balance of total dissolved solids (TDS)

Centrifugation removed primarily lignin and lignin-related substances which were precipitated by cooling of the water extract to room temperature after the extraction. However, part of the TDS was not accounted for after the fractionations. Most of the unrecovered materials were left on the membranes, especially on the membrane (300 kDa) which was observed visually (Figure 41). The main fraction of TDS was in the fraction (30 – 100 kDa), about one third of the TDS after centrifugation. The fractions (10 – 30 kDa) and (< 3 kDa) were also relatively large.

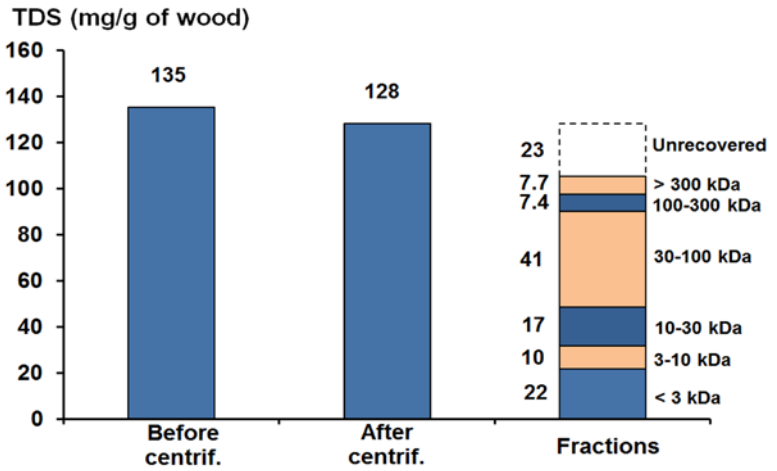


Figure 41. Total dissolved solids in different fractions.

The amount of precipitated wood materials had a maximum yield when the water content in ethanol-water solution was 10%, amounting to about 80 mg/g of wood, which was about 60% of the total yield after centrifugation (Figure 42).

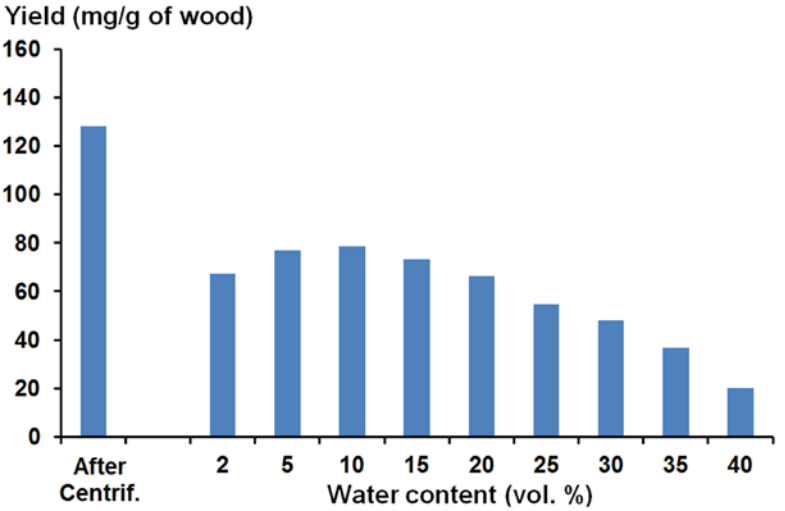


Figure 42. Total yields of precipitated material in ethanol with different water contents.

The yield of precipitated material decreased with increased water content.

Total non-cellulosic carbohydrates and average molar mass

About 27% of the polymeric and oligomeric non-cellulosic carbohydrates in the starting raw extracts were also removed by centrifugation. However, the composition of the extracts before and after centrifugation was quite similar (Figure 43).

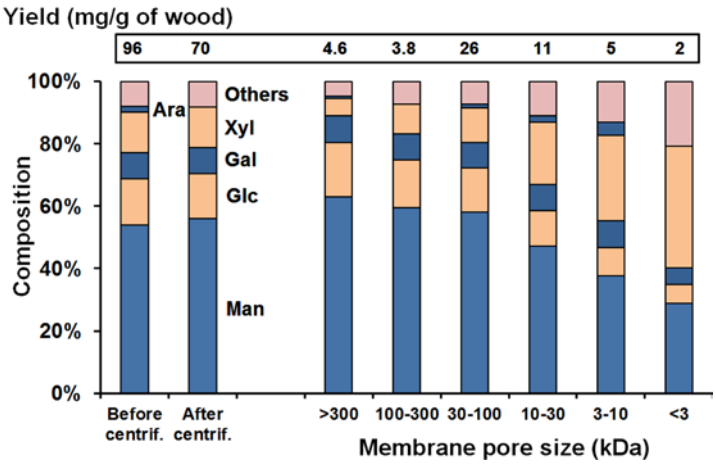


Figure 43. Total amount (as anhydro sugars) and composition of polymeric and oligomeric non-cellulosic carbohydrates in different fractions.

Same to TDS results, the fraction (30 – 100 kDa) was abundant in dissolved polymeric and oligomeric non-cellulosic carbohydrates and the molar mass was distributed mostly in the range of about 4 – 15 kDa with an average Mw about 5.5 kDa (Figure 44).

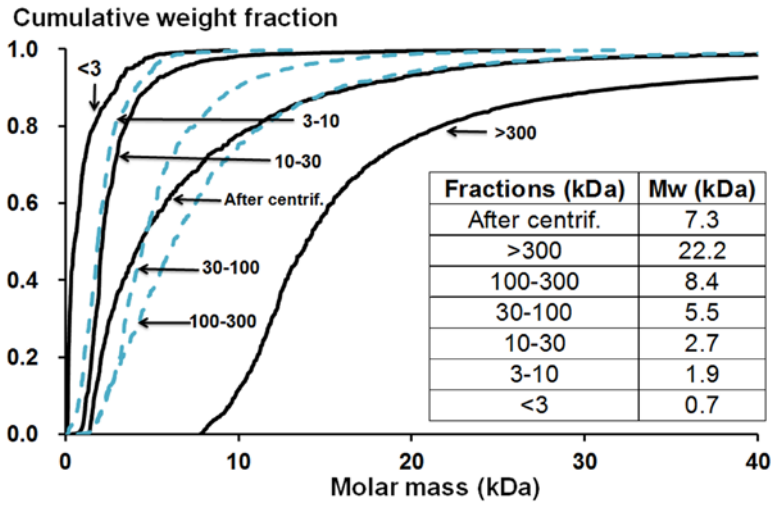


Figure 44. Mw distribution and average Mw of non-cellulosic carbohydrates in different fractions.

There were also considerable amounts of polymeric and oligomeric non-cellulosic carbohydrates in the fraction (10 – 30 kDa), but both the yield and average Mw were lower than for the fraction (30 – 100 kDa). The fraction (> 300 kDa) had the highest average molar mass, about 22 kDa, in the range of about 8 – 35 kDa. However, the yield of this fraction was very low.

GGM-derived mannose, glucose and galactose units dominated especially in the high-molecular fractions. However, with the decrease of the membrane pore size, and consequently also decrease in molar mass, the portion of xylose, arabinoses, and others (mainly rhamnose and uronic acids) increased.

The yields of precipitated non-cellulosic polysaccharides were highest when the water content was 5 – 15% (Figure 45).

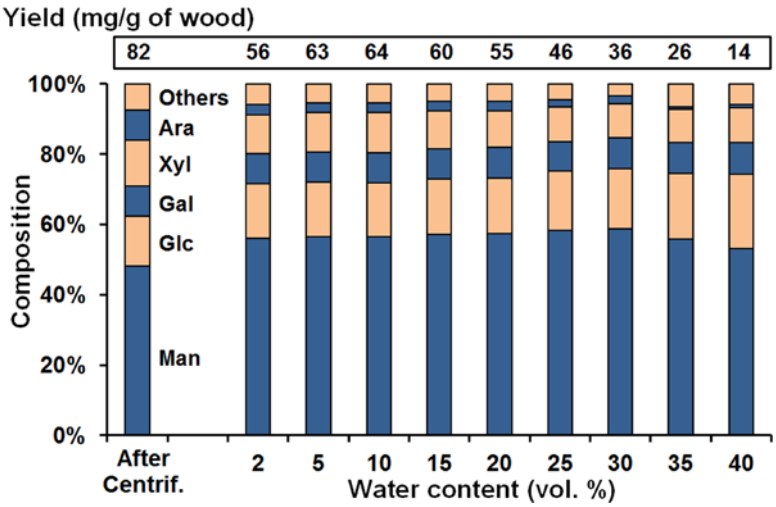


Figure 45. Composition and yield of non-cellulosic carbohydrates precipitated in ethanol with different water content.

GGMs were the main polysaccharide in all precipitates. The highest yield of precipitated non-cellulosic carbohydrates was obtained with 10% water content, with the value 64 mg/g of wood, corresponding to 78% of total non-cellulosic carbohydrates in the extract after centrifugation.

The precipitate obtained with the highest tested water content (40%) had the highest Mw (Figure 46), about 22 kDa in the range of about 5 – 40 kDa. The Mw increased with the increase of water content higher than 10%.

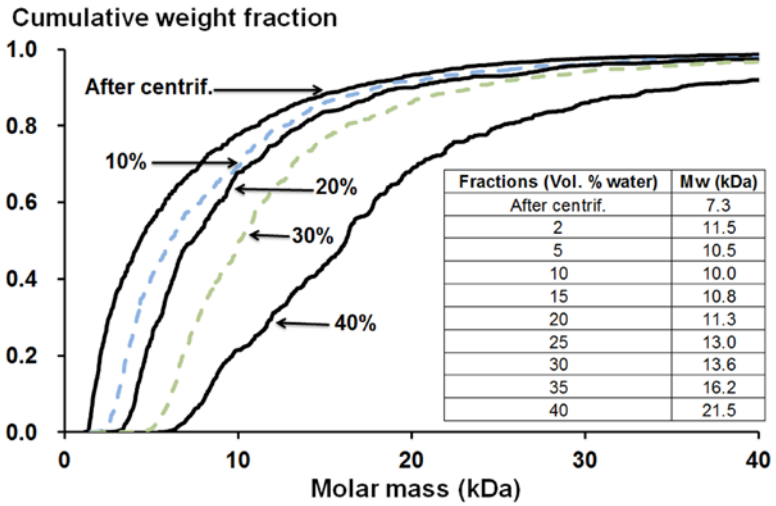


Figure 46. Mw distribution and average molar mass (Mw) of non-cellulosic carbohydrates precipitated in ethanol with different water content.

The polysaccharides in all precipitates had a similar sugar units composition as in the extract after centrifugation. However, it is obvious that GGMs are precipitated to a higher extent than xylans, probably because GGMs are more stable against acid hydrolysis and thus have a higher molar mass. GGM-derived mannose, glucose and galactose comprised about 80% of the sugar units, with a molar Man:Glc:Gal ratio of 3.6:1.0:0.6.

Acetic acid and acetyl groups

Almost all free acetic acid, released during the ASE extraction, was in the fraction (< 3 kDa) (Figure 47). In the other five fractions acetic acid was present in esterified form as acetyl groups, which are attached to mannose units in GGMs. In spruce wood the degree of GGM acetylation, calculated as the molar Ac/Man ratio, is about 0.50. With decreasing molar mass the degree of acetylation decreased slightly, but was still above 0.40 even in the low-molar fraction (3 – 10 kDa).

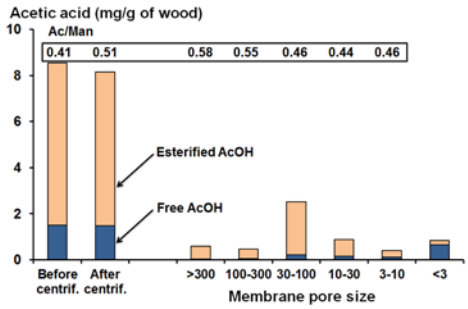


Figure 47. Amount of acetic acid and degree of acetylation (Ac/Man) in different fractions.

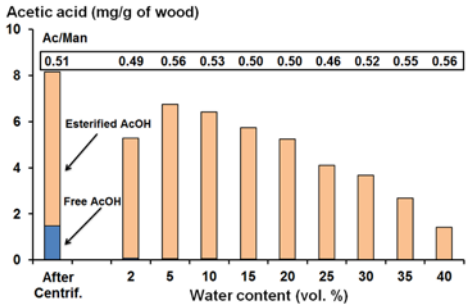


Figure 48. Amount of acetic acid and degree of acetylation in different precipitates.

All precipitated non-cellulosic polysaccharides had a degree of acetylation in the range of 0.46 – 0.56, which is around the degree of acetylation of native GGMs in spruce wood (Figure 48).

Mass balance of lignin and lignin-related substances (LLRS)

About one third of the LLRS extracted from wood was removed after centrifugation, being about 7 mg/g of wood (Figure 49). Most of the LLRS was present in the fraction < 3 kDa. The LLRS in the other fractions was probably, as for the monosaccharides, left in the remaining water on the membranes.

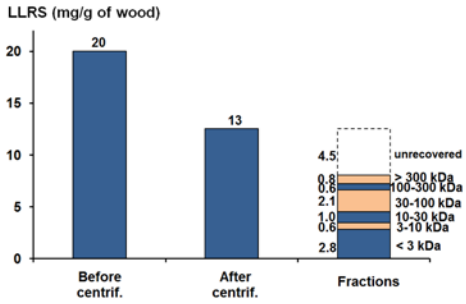


Figure 49. Amount of lignin and lignin-related substances in different fractions.

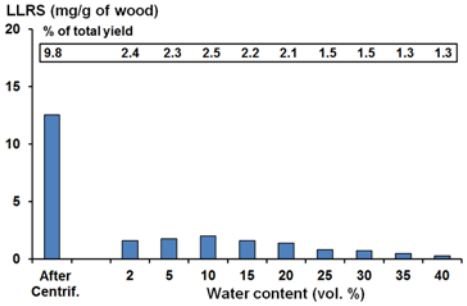


Figure 50. Amount of lignin and lignin-related substances in different precipitates.

LLRS is precipitated with ethanol-water to a much lower extent than non-cellulosic polysaccharides, and the precipitates thus contain much less of

LLRS (Figure 50). The residual small amounts of lignin may be part of lignin-carbohydrate complex (LCC) in the precipitates (Lawoko et al., 2006). The lignin yield, as well as the portion of lignin in the total yield, decreased with the increase of water content over 10%.

Concluding remarks

Purification of hot-water extracts of spruce wood to produce pure GGM-rich polysaccharides can be achieved both by membrane filtration and precipitation in aqueous ethanol. Precipitation in ethanol separates exclusively polysaccharides, mainly GGMs, with a lower molar mass limit of about 4 kDa, corresponding to a degree of polymerisation (DP) of about 25. The highest yield is obtained with 10 – 15% water content. With higher water content the yield will be lower but the molar mass will be higher. Very little lignin is precipitated and the precipitate is a white powder. GGMs are precipitated to a higher degree than xylans and pectins.

With membrane filtration it is possible to obtain not only a polymer fraction but also fractions rich in oligosaccharides and monosaccharides. However, the obtained fractions are not so pure, containing more lignin and lignin-related material, than ethanol-precipitated fractions.

Membrane filtration is an established separation technology that is successfully applied in many industrial processes. Precipitation with ethanol-water is convenient in laboratory conditions. However, in industrial conditions ethanol precipitation can be rather expensive when a large volume of ethanol has to be recovered by distillation. Pre-concentration of the water extracts with membrane filtration can reduce the ethanol demand for precipitation.

3.3 Structural characterisation of galactoglucomannans by NMR

The structure of the purified GGM-rich precipitate was characterised by ^{13}C NMR spectroscopy (Figure. 51).

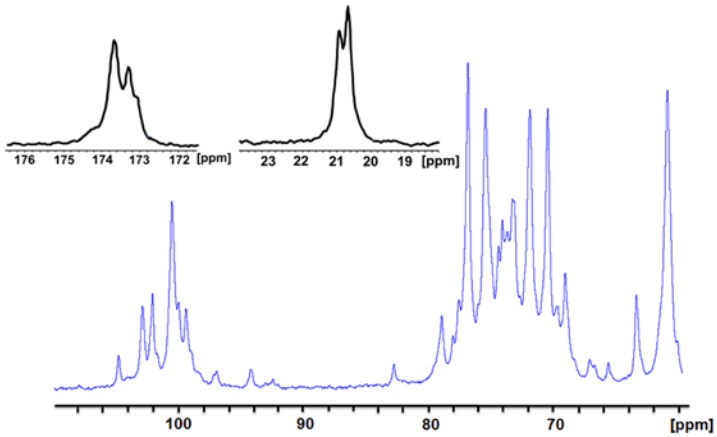


Figure 51. NMR spectrum of purified GGMs precipitated in water:ethanol (15:85).

The ^{13}C NMR spectrum shows signals from different carbonyl carbons as well as methyl carbons assigned to acetyl groups (δ = 170 – 180 ppm, and 15 – 22 ppm, respectively). Carbonyl groups in lignin may also appear in the range of 170 – 180 ppm (Willför et al., 2003). There were also signals of different C1 – C6 carbons (δ = 50 – 110 ppm).

The spectrum is almost identical to that of GGMs extracted from spruce wood with water at 90°C presented by Willför et al. (2003). It is obvious that the GGM-rich precipitate obtained by extraction of spruce wood at 170°C for 20 minutes is very similar to that extracted at milder conditions, at 90°C for 1 h.

3.4 Kinetic study of galactoglucomannans degradation in hot water

The degradation kinetics of isolated high-molar-mass GGMs was studied by treatment in hot water adjusted with phthalate buffers to three pH levels. The study was made at 170°C for up to 90 min, similar to typical hot-water extraction conditions.

The hydrolysis of the isolated GGMs, also containing xylans and pectins as

impurities, was studied using the same ASE apparatus and the same phthalate buffer solutions as in the extractions, except the pH 4.4 solution. In these experiments with a rather low GGM concentration, the pH decreased only slightly even during 90 min treatment. The amounts of monosaccharides released during treatment for 60 min at 170°C are shown in Figure. 52, and the average molar mass of GGMs and GGM-derived products (Figure 53) was calculated by ASTRA software after analysis by HPSEC-MALLS.

Monosaccharides

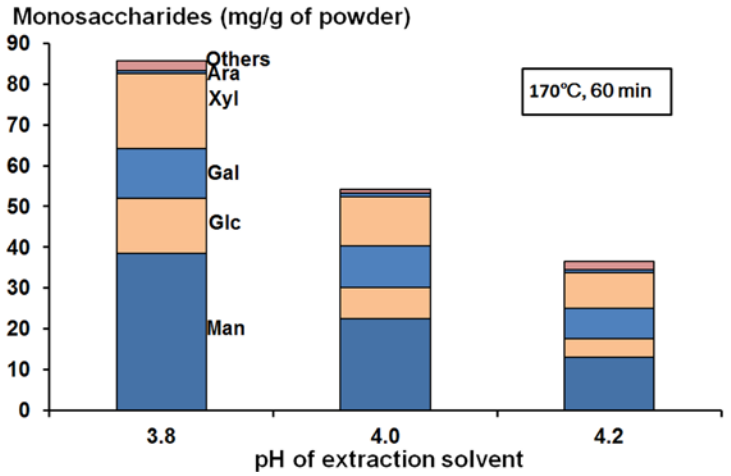


Figure 52. Monosaccharides released by treatment of model GGMs in different phthalate buffers at 170°C for 60 min.

The hydrolysis of GGMs to monosaccharides decreased clearly in the narrow pH range 3.8 – 4.2. The relatively high amount of released xylose is explained by the lower hydrolytic stability of xylans compared to GGMs (Abatzoglou and Chornet, 1998).

Average molar mass (Mw)

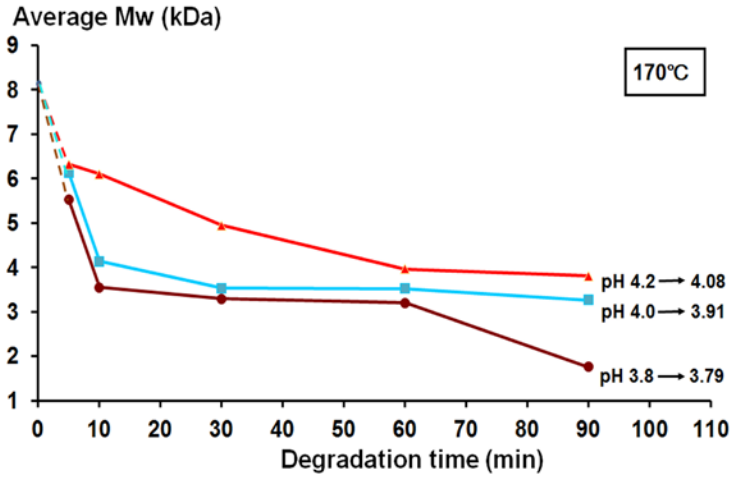


Figure 53. Decrease in average molar mass of model GGMs in different buffer solutions.

The molar mass results verify the strong effect of pH in the studied narrow pH range. A higher pH resulted in higher average molar mass of final extracts. At lower pH (3.8 and 4), the molar mass of extracted GGMs dropped extensively, from 8.5 kDa to about 4 kDa after 10 min. Higher pH phthalate buffer 4.2, however, inhibited the degradation of GGMs more than lower pH. The molar mass dropped slowly from 8.5 kDa to about 6 kDa after 10 min.

With the developed kinetic model, the degradation of GGMs was well predicted (Figure 54).

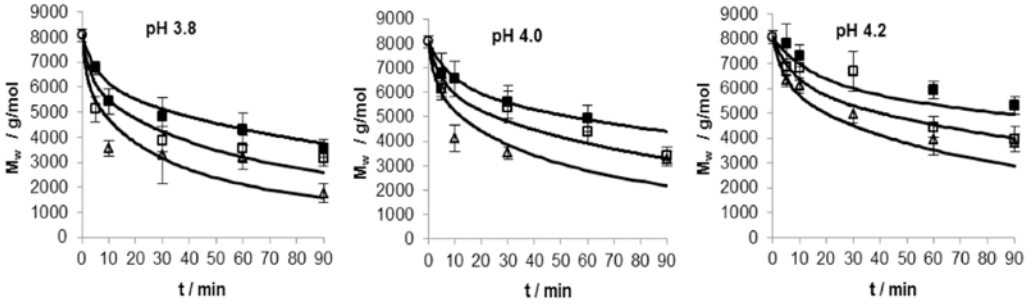


Figure 54. The lines represent the predicted values and the dots represent the measured values. Relative errors are shown for the measured values. The symbols refer to (○) initial material and temperature of the experiments: (■) 150, (□) 160, and (△) 170°C (VI).

The model fits quite well the evolution of the number and weight average molecular weight of the GGMs in the hot-water extraction conditions when all the glycosidic bonds break at the same rate (VI). The obtained kinetic parameters were in good agreement with the earlier results obtained for the degradation of linear mannans during homogeneous thermal hydrolysis.

4. CONCLUSIONS

The main focus of this work was on extraction of galactoglucomannans in polymeric form in high yield from spruce wood with pressurised hot water.

Pressurised batch extraction using an accelerated solvent extractor (ASE) apparatus is a practical and convenient means for extraction research. Both chips and ground wood can be extracted using ASE, with pure hot water or with water solutions containing added chemicals. With an ASE apparatus it is possible to perform repeatable extraction experiments under accurately controlled conditions regarding temperature and time. Furthermore, a large number of samples can be extracted conveniently in parallel.

Pressurised hot-water extraction (PHWE) of ground spruce wood with plain water in the temperature range of 160 – 180°C can give a total extraction yield up to 25% of wood, with preferential extraction of the main hemicellulose type, the partly acetylated galactoglucomannans (GGMs). Xylans are extracted to a lower extent. Lignin and pectins are also extracted to a lower extent than GGMs. The extraction yield increases with the temperature and extraction time. For comparison of different extraction conditions in technical processes it can be useful to combine these two main parameters into one, such as the P factor. However, the P factor does not take into account the strong influence of pH, and moreover, the process chemistry can be different at different temperatures, even if the P factor is the same.

During PHWE two hydrolytic reactions dominate: (i) cleavage of acetyl groups (deacetylation) and (ii) cleavage of glycosidic bonds. Deacetylation lowers the solubility of polymeric GGMs, while glycosidic bond cleavage shortens the chain length leading to easily soluble oligo- and monosaccharides. pH is a critical parameter governing the extraction of GGMs in polymeric form which was the main aim of this work. pH of the extracts changes during PHWE, and drops with the increasing temperature and time. A pH of 3.5 or lower, leads mainly to oligo- and monosaccharides, and further even to degradation of the monosaccharides. On the other hand, a high pH, 4.5 or higher, leads to extensive deacetylation and consequently to a

low solubility of polymeric GGMs.

Keeping the pH around 4, for example with buffers, can improve the extraction of polymeric GGMs, and extraction in good yields (up 8% of wood) demands appropriate control of pH to that level.

Extraction of polymeric GGMs is restricted by the diffusion out of the S2 wall and further out of the wood chips or particles. The GGM yield from chips is only about 60% of that from ground wood with a particle size below 1 mm. Very small wood particles (< 0.1 mm) give 20 – 40% higher yields than larger particles, in the range 1.25 – 2 mm. However, grinding of wood to small particles consumes energy and lowers the fibre properties with respect to possible pulp and paper production. There are only small differences in extraction yields between different spruce wood materials, such as sapwood, heartwood and TMP.

Polymeric GGMs can be separated from hot-water extracts by precipitation in ethanol and/or membrane filtration. Precipitation in ethanol separates exclusively polymeric hemicelluloses with a molar mass above 4 kDa. The highest yield of polymeric GGMs is obtained with water content of 5 – 15%. A higher water content gives precipitation of GGM of higher molar mass, although in a lower yield.

Membrane filtration enables separation also of fractions rich in oligosaccharides and monosaccharides. However, the obtained fractions are not as pure as those from precipitation in ethanol. In industrial conditions ethanol precipitation can be rather expensive when a large volume of ethanol has to be recovered by distillation. Pre-concentration of the water extracts with membrane filtration can reduce the ethanol demand for precipitation.

PHWE can also be seen as the first stage in a concept for extraction of the three main components in wood, i.e. hemicelluloses, lignin and cellulose. Mild conditions, especially by avoiding a too low pH, in PHWE is probably beneficial also for the consecutive extraction of lignin in conventional or new cooking processes.

The obtained large body of experimental data could be utilised for further kinetic and economic calculations to optimise hot-water extraction of softwoods.

For further development of production of polymeric GGMs, technical PHWE extraction processes should be developed where pH can be maintained at the level of 4, by using suitable buffers or other pH control. Combinations of membrane filtration and solvent precipitation techniques should also be developed. However, a key target for future research is to identify technically and commercially viable applications for GGMs. Now, polymeric GGMs can be produced in sufficient amounts for such application research and development.

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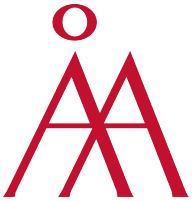

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